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AFSTI

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REVIEW

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Functional Properties of Grain Legume Flours

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Grain legumes as foods are favoured because of their nutritional quality, palatability and versatility in food preparations. In recent years, researchers have emphasized expanding the utilization of grain legumes in the form of meal and flavour for use as functional ingredients. Several important functional properties such as water and oil absorption, flour solubility and swelling power, foam capacity, emulsification, gelation capacity, gel strength, gel consistency, paste viscosity, gel temperature and nitrogen solubility index (NSI) of grain legume flours have been discussed in this review, keeping in view, the available information on their food applications. The possible future areas of research to improve the end-product quality of grain legume have also been indicated.

Keywords : Legume flours, Functionality test, Methodology, Significance, Variability, Processing factors.

Man has always selected the types of food crops with some consideration for their nutritional and functional attributes, although these have not been properly understood. In this context, grain legumes, which are interchangeably called pulses in the Indian sub-continent have occupied an important place in the daily diets of the population of the region due to their nutritional potentials, particularly as rich sources of proteins. Attention has been paid worldwide to improving the nutritional quality of grain legumes. The supplementation of cereals with high protein legumes is considered to be one of the best solutions to the protein-calorie malnutrition, particularly in the developing countries. It is often emphasized that considerable research attention is needed to improve the production and nutritional aspects of grain legumes, which are commonly grown and consumed by humans. These include dry bean (*Phaseolus vulgaris*), pigeonpea (*Cajanus cajan* L.), cowpea (*Vigna ungulculata*), chickpea (*Cicer arietinum* L.), broad bean (*Vicia faba*), dry pea (*Pisum sativum*), soybean (*Glycine max*) and groundnut (*Arachis hypogea*) accounting for about 95% of the total world legume production (Singh et al. 1997).

In view of the increasing utilization of grain legumes in composite flours for various food formulations, their functional properties are assuming greater significance. The functional characteristics provide a set of data, which gives information on the fields of application in food formulations (Hermansson 1979). These can be used as a guideline in product development, especially in wheat-legume composite flours, where proteins are used as major functional ingredients (Fernandez and Berry 1989). Very often, researchers have emphasized expanding the utilization of grain legumes in the form of meal and flour for use as functional ingredients. The functional properties are provided not only by the proteins of flours, but also by complex carbohydrates and other grain components such as pectins and hemicellulose (Martinez 1978). In recent years, functional foods are assuming greater importance and have attracted the attention of food processors, marketers and consumers (Reilly 1994).

Voluminous literature exists on the nutritional composition of grain legumes. This includes protein and carbohydrates vitamins, minerals and antinutritional factors of grain legumes (Singh an Singh 1992). But, only limited information is available on the functional properties of grain legumes. Moreover, no review on the functional properties of grain legumes has appeared in the recent past. The available information on various functional properties of common interest of different legumes have been summarized under suitable headings in this review. The areas of future research are also highlighted with special reference to product development and end-use quality of grain legumes.

Definition of functional properties

Functional properties are physico-chemical properties, which give information on how a particular ingredient (e.g. protein, carbohydrate) will behave in a food system. The functional properties of plant foods are determined by the molecular composition and structure of the individual component and their interactions with one another (Kinsella 1976).

About functional properties

The terms most appropriate to functional characterization have been used and described by researchers in the past for the purpose of their being employed for various food formulations. The following functional properties, which are generally referred and have found wider applications in food formulations have been discussed in this review.

i) Bulk density; ii) Water and oil absorption; iii) Flour solubility and swelling power; iv) Foam capacity; v) Emulsification; vi) Gelation capacity; vii) Gel strength; viii) Gel consistency; ix) Paste viscosity; x) Gelatinization temperature; and xi) Nitrogen dispersibility index (PDI).

Methods of determination

A great deal of emphasis is always given to the determination of functional tests. Reliability and reproducibility of analysis are the major considerations in employing the methods of determination of various functional properties. Depending on the availability of facilities and expertise, these characteristics are evaluated by different researchers and food processors. A brief and simplified version of these methods is desirable and described for the benefit of uniformity of determinations by different researchers.

Bulk density : The simple method described by Narayana and Narasinga Rao (1982) is generally used to determine bulk density. A calibrated centrifuge tube is weighed and the sample is filled to 10 ml mark by constant tapping, until there is no further change in volume. The content is weighed and from the difference in weight, the bulk density of the sample is calculated.

Water and oil absorption : The determination of water and oil absorption involves the most simple procedures, which produce reliable and dependable results. Generally, 1 g sample is mixed with 10 ml of distilled water or 15 ml oil for 30 min. The contents are allowed to stand at 30°C in a water bath for 30 min and then centrifuged at 3000-5000 rpm for 20 to 30 min, depending on the availability of facility for this purpose. After centrifuging the content, the volume of the supernatant is recorded and used for determination of water and oil absorption and the results are expressed as g/g sample. The water and oil absorption methods have been described by Rosario and Flores (1981).

Flour solubility and swelling power : These characteristics are generally determined at temperatures, when the process of gelatinization of starch granules takes place. A convenient and reliable procedure for determining flour solubility was recently standardized and described by Iyer and Singh (1997). According to this procedure, 250 g finely ground defatted flour is thoroughly mixed with 15 ml of distilled water and heated to 65°C, being the initial temperature at which gelatinization of starch granules begins. The content is then cooled to room temperature and centrifuged at 5000 rpm for 10 min. The soluble solids content is calculated as percentage of flour soluble in water. At the same time, this can be used to calculate the swelling power, since the sample has been heated at 65°C for 30 min. The starches of some grain legumes may follow two-stage swelling pattern and in such situations, heating at 95°C is also suggested. After heating and centrifuging as described above, the residue is weighed and increase in weight is calculated as the swelling power of the sample at that particular temperature.

Foam capacity : The foam ability of legume flour is a desirable characteristic for the production of some traditional foods. This is generally determined at pH 7.0 as described by Coffman and Garcia (1977). The volume of foam after whipping is expressed as the foam capacity and the volume of the foam over a time period 10-120 min is expressed as the foam stability for the respective time periods. Foam capacity measurements are also made using NaCl solution of 0.2-1.0 M concentrations.

Emulsification : The oil-in-water emulsions formed are taken as an index of the emulsification capacity. The emulsions are generally prepared according to the method by Sathe and Salunkhe (1981). According to this method, the sample (2 g) is blended in a Waring blender with 100 ml distilled water for 30 sec at high speed. The oil is added in 1 ml or 0.5 ml portions as per the requirement. The amount of oil added up to the point where a fine layer of oil is formed, indicating that no more oil is required by the sample suspension. This is recorded as the emulsification value or the emulsification capacity of the sample.

Gelation capacity : By and large, the method described by Rosario and Flores (1981) is followed for the determination of gelation capacity. Sample suspensions containing 8 to 12% (w/v) flour in 0.5% increments are prepared in 10 ml of distilled water. Then, the test tubes are heated for 1 h in boiling water, rapidly cooled under running cold tap water and refrigerated for 3 h at 5°C. The least gelation concentration is that concentration at which the sample does not fall down or slip from an inverted test tube. It may be emphasized that the quantity of sample for preparing suspension would depend on the gelation capacity of the legume flours and some may require higher quantity than the others.

Gel strength : The gel strength is generally measured using a compression cell (0-5 kg full scale) in an Instron Food Testing Machine (IFTM). The gels are formed first and then compressed. For forming gels, the isolated starch samples (6% starch) are heated at 95°C for 10 min, poured into a round moisture dish (5 cm diameter) and stored at room temperature for 12 h. Thus, uniformly polymerized round starch gel slabs of about 1 cm thickness are removed from the moisture dish and compression force is measured by pressing gel slab between two flat plates, considerably larger than the sample slab, so that no puncture takes place.

Gel consistency : The gel consistency is determined by boiling the sample in distilled water and then cooling it to a desirable consistency. The spread of the thus formed gel is recorded as the gel consistency of the sample. The modified method by Iyer and Singh (1997) appears to be convenient and reliable for this purpose. According to this method, distilled water (10 ml) is added to a finely ground sample (0.5 g) in a beaker. The suspension is boiled for 20 min and after boiling, the material is taken in a petri dish of known diameter and depth (40 ´ 10 mm). The material is cooled to room temperature and transferred to a smooth glass surface by inverting the petri dish. The petri dish is slowly removed and the diameter of the gel that spread out on the glass is measured as gel spread and expressed as gel consistency of the sample.

Paste viscosity : There is also called as hot paste viscosity or peak viscosity (Iyer and Singh 1997). Depending on the concentration of the starch in flour sample, viscosity values are commonly obtained with a Brabender viscoamylograph using 6-8% starch concentration as per Lii and Chang (1981).

Gelatinization temperature : This is the temperature at which the starch granules get gelatinized and stain with congo-red. The most commonly used method for determining the gelatinization temperature is similar to that followed for cereal starches. The gelatinization temperature of starch is determined by using a light microscope and congo-red (0.2%) is used as a stain. Singh et al (1989) described a slightly modified method for determining the gelatinization of legume starches. According to this procedure, an aqueous solution of flour (starch) is heated using a mini block heater and samples are taken from 60°C onwards at 1°C intervals, until the gelatinization temperature is reached. Starch granules are

stained at initial, mid point (50% stained) and final (90% stained). The temperature at which 90% of the starch granules are stained is recorded as the gel temperature of the sample.

Nitrogen solubility index (NSI) : The NSI is a function of the pH of the extracting solvent. The higher concentration of nitrogen is soluble in acidic and alkaline pH ranges. The NSI is generally determined at pH 6.8-7.0 according to AACC(1983) method. The pH of the extracting solvent is adjusted by using dilute acid or alkali and the soluble nitrogen in the supernatant is determined by Kjeldahl method.

What does contribute to functionality : The protein and starch are the principal constituents of grain legumes, since they serve as reserves of storage organs of plant tissues used for food by humans. The most important aspects of vegetable proteins are their functional properties (Hermansson 1979). Sometimes, functional properties are determined in order to predict the behaviour of protein ingredients. Proteins take part in a very complex food structures and hence perform a specific function in foods.

Properties of proteins contributing to the functionality of plant foods have occupied an important place and remained a point of focus of attention of food scientists and nutritionists (Wall 1979). Total proteins have been reported to be primarily responsible for increased water retention in foods (Hutton and Campbell 1981). According to these workers, the protein-water interactions occur at polar amino acid sites on the protein molecules. Most proteins contain numerous polar side chains along their peptide backbone, making them hydrophilic. Hydration of proteins is vital for several of the functional properties such as emulsion capacity, foaming, viscosity and gelation (Sathe and Salunkhe 1981). The role of proteins in determining several functional properties is summarized in Table 1.

In addition, the functional properties are provided by complex carbohydrates and other components such as pectins and mucilages. The starch components are used as valuable ingredients in the food industry because of their versatile functional properties. The water absorption during cooking is a function of the starch granules, which are held together by hydrogen-bonding forces in the form of crystalline bundles, called micelles (Schoch and Maryland 1968). When an aqueous suspension of starch granules is heated, these structures are hydrated and eventually sieving takes place, making it an inherited characteristic of starch granules (Singh et al. 1989).

TABLE 1. FUNCTIONAL PROPERTIES OF PROTEINS AND THEIR PRACTICAL APPLICATIONS IN VARIOUS FOODS

Property	Applications
Emulsification	Salad dressing, coffee whiteness, meats
Hydration	Doughs, meats
Viscosity	Beverages, doughs
Gelation	Sausages, gel desserts
Foaming	Cakes, toppings
Cohesion binding	Textural products, doughs
Textural properties	Textured foods
Solubility	Beverages

However, starch is not a good emulsifying agent primarily because it lacks polyelectrolyte character necessary for emulsion stability (Pomeranz 1991). This review discusses that the presence of carbohydrates and fibre in legume and cereal flours adversely affects the emulsion capacity. Carbohydrates other than starch also deserve consideration while evaluating the functional properties of various foods. Susheelamma and Rao (1979) reported the role of arabinogalactan (a polysaccharide) of blackgram in the texture of leavened foods. An overview of starch structure, as it relates to several of its functional properties has previously been covered by Seib and Marta (1992). Starch functions as an adhesive binder, encapsulating agent film former, gelling agent, water binder, texturizer and fat paring agent, with numerous other applications both in the food and non-food areas (Mauro 1996).

Role of functional properties

Since the consumer's choices for foods are expanding, the food, industries are increasingly depending on the ingredients, which can provide good functional and nutritional properties to the food being marketed. Particularly, the functional properties are required for the formulation of various value-added food products.

In the first place, water is the most important component of food, which plays an important role in the major changes that take place during the cooking of foods. More importantly, water absorption of legume grains particularly of the decorticated ones is directly related to the cooking characteristics and is extremely important in determining all cooking behaviour. Water absorption helps separation of the cells in the cotyledons of legumes. The cell separation, in turn, helps cooking process (Rockland and Jones 1974). The extent to which pulse grains or *dhals* absorb water on soaking is directly related to the cooking time (Singh 1999). This characteristic has also a greater application in maintaining the soft texture cookies of cereal-legume composite flours. Proteins are important in maintaining moisture in semi-moist foods and baked cereal foods (Wall 1979). In this context, protein functionality of grain legumes could play an important role in value-added bakery products of cereal-legume composite flours. For example, the soft textured cookies could be prepared by using soyflour as an ingredient, which would enhance the water holding capacity during the baking process, thus giving the finished products with a higher than usual moisture content and a soft texture. Water absorption characteristics represent the ability of a product to associate with water under conditions where water is limiting in doughs and pastas. Some studies have suggested that the raw and heat-processed legume flour could be useful in food systems such as bakery products, which require hydration to improve handling characteristics (Giami 1993). Hydration properties, dispersibility, water absorption, binding, swelling and viscocity are known to directly influence the characteristics of a food system (McWatters 1983). In addition to water absorption capacity, the fat absorption capacity is also important, as it improves the mouthfeel and retains the flavour (Kinsella 1976).

Protein dispersibility index (PDI) is an important parameter of specification of legume flours for use in bakery as supplements to cereal flours. Utilization of food ingredients, especially high protein materials, depends to a large extent on functional properties. Nitrogen solubility profiles over a pH range are being used increasingly as a guide to protein functionality. Some correlations have been found between functional characteristics of soy flour in various applications and nitrogen solubility index (NSI). Generally, the higher the water dispersible protein in a soy protein concentrates, the better its emulsifying action (Victor and Inglett 1974). Whipping properties of soybean products expressed by foam expansion and foam stability, correlated with water-dispersible nitrogen (Yasumatsu et al. 1972). Easily dispersible proteins of legumes with those of cereals in a mixed diet could enhance the digestibility of cereal-legume proteins of mixed diets. Among the functional properties of proteins, solubility is probably the most critical because it affects other properties such as emulsification, foaming and gelation (Kinsella 1976). The protein solubility as influenced by heat processing due to denaturation of protein plays a significant role in determining the digestibility of the legume proteins.

The extent to which the functional properties undergo changes as a result of heat treatment could help in estimating the requirement of heat for processing legumes for various food formulations (McWatters and Holmes 1979). According to this study, thick salad dressing-like emulsions were produced by soy flour heated for 30 min and peanut flour heated for 20 min. The viscosity of emulsions produced by soy flours has some applications in meat and bakery products (Kinsella 1979). Viscous emulsions are formed as a result of heat treatment and these could provide a good indicator for the amount of heat required to process the legume flours for human consumption (McWatters and Holmes 1979). It was reported by these workers that less heat may be required to process the peanut flour as compared to soy flour because less reduction in emulsion capacity is noticed in the former as a result of heat processing. In other words, higher percentage of reduction in emulsion capacity due to heating would indicate that higher amount of heat would be required to process the flour for human consumption.

The starch granules of pulses have greater stability against the mechanical shear than those of the fragile swollen wheat starches because of the hot paste viscosity that does not show any break down point in pulses (Iyer and Singh 1997). The visicosity patterns of starches are primarily determined by the extent of swelling of the starch granules and by the resistance of the swollen granules to the dissolution by heat or fragmentation by the shear force. Generally, legume starch pastes are more viscous than those of cereal starches, indicating that legume starches have a higher resistance to swelling and rupture than do cereal starches (Lineback and Ke 1975; Lai and Variano-Martson 1979). These properties are very vital for making transparent noodles of legume starches, which are becoming very popular in the orient (Singh et al. 1989).

In a very investigation, the ability of pre-heated flours to form a viscous paste and to form a gel upon cooling was reported to be due to protein gelation rather than starch gelatinization in legumes (Prinyawiwatkul 1997). The gelation capacity and gel firmness in both legumes and cereals may also be contributed by protein molecules (Singh and Singh 1991). A specific functional property may be essential for making a particular food product (McWatters 1980). It was suggested that cowpea flour was suitable in viscous foods like gravies, soups, baked products but may not be suitable for foods like *idli* and *dosai,* where good foaming properties are essential (Padmashree et al. 1987). The foamability of cowpea flour was found to be a desirable characteristic for the production of several traditional cowpea-based food products in Nigeria e.g. soups and stews (McWatters 1983).

The presence of amylose and amylopectin in different proportions in different legumes might attribute to their cooking behaviour and eating quality (Pomeranz 1991). Generally, varieties with the highest amylose contents show the lowest peak viscosities in cereals. But this has not been clearly understood in legumes as to how does the variation in amylose content influence viscoamylographic properties. It becomes increasingly apparent that the non-amylose fraction, amylopectin of rice starch may be more influential in cooking behaviour (Pomeranz 1991). However, the role of amylose and amylopectin in cooking behaviour of grain legumes needs to examined in greater details.

The preparation of Chinese-type noodles with defatted peanut flour and cowpea flour, alone and in combination with wheat was possible because of compatible functional properties (McWatters et al. 1995). According to this study, noodle firmness was affected primarily by peanut flour. In functional characterization, a set of data is obtained, giving information on the fields of application for a certain protein ingredient. This is especially important for charcterization of gels, emulsions and mixtures of structures for the development of sausage formula (Hermansson 1979). The biscuit doughs containing the air-classified proteins of fieldpea were less sticky than those of the reference doughs and this was attributed to the functional properties of fieldpea proteins (McWatters 1980). The ability of cowpea flours to absorb and retain water and oil may help improve binding of the structure, enhance flavour retention, improve mouthfeel and reduce moisture and fat losses of extended meat products (McWatters and Heaton 1979).

The starches used in baking contribute to structure, increase batter viscosity and cake volume, control moisture and increase shelf life (Mauro 1996). Further, pre-gelatinized starches offer some distinct advantages in cakes and muffins. The gelatinization characteristics have been shown to influence the cooking characteristics of rice varieties (Lii and Chang 1981). This aspect has not fully examined in grain legumes, particularly in view of the fact that legume flours are good supplements to cereals in making value-added products.

The bulk densities of legume flours play a key role in the formulation of weaning foods (Malleshi et al. 1989). It may be expected that decreased bulk density would be an advantage in the preparation of weaning food formulations.

TABLE 2. VARIABILITY IN FUNCTIONAL PROPERTIES OF SOME GRAIN LEGUME RAW (UNPROCESSED) FLOURS[a]

Legume	Water absorption, ml/g	Oil absorption, ml/g	NSI at pH 6.5, %	Gelatin capacity, %	Emulsification capacity, ml/100g	Gelatinization temperature, °C	Hot paste viscosity (Bu)
Soybean[b]	0.80-0.95	1.0-1.10	45.5	9.5	28.0-35.60	68.0-72.0	410
Drybean[c]	1.67	1.0	62.0	-	39.6	69.0	-
Chickpea	0.92-1.10	0.92-1.55	51.4-63.7	9.0	3.1-3.8	73.0-75.0	320-410
Pigeonpea	0.90-1.30	0.85-1.40	48.0-72.6	8.5	-	76.0	302
Mung bean	0.79	0.84	52.0-68.5	10.0	8.6-17.0	72.0	315
Winged bean[c]	2.10	2.20	48.0	-	25.5	70.0	-
Cowpea	0.90	1.40	55.6-70.0	-	32.0	-	-
Field pea	0.85-1.20	1.05	46.4-65.6	-	14.5-23.0	68.0-70	455
Horsegram[c]	0.92-1.15	0.87-1.00	-	-	28.8-31.7	-	-

[a]Values on decorticated samples compiled from different sources/references, Bu=Brabender unit. [b]Partially defatted flour (PDF), [c]On the basis of whole seed samples

TABLE 3. FUNCTIONAL PROPERTIES OF *DESI* AND *KABULI* CHICKPEA FLOURS

Genotype	Water absorption, g/g	Oil absorption, g/g	Emulsification capacity g/g	Emulsification capacity g/g protein	Swelling capacity 65°C	NSI, %	Hot paste viscosity, Bu
Desi (Armethyst)	0.92	0.92	3.84	15.31	4.70	63.5	32.0
Kabuli (Garnet)	1.00	1.55	3.13	14.93	2.67	49.5	41.0

Source : Iyer and Singh (1997). Bu = Brabender units

Variability in functional properties among legume species

Grain legumes exhibit great diversity in terms of their origin and the environments wherein they are grown. Depending on the legume species and methods of analyses, large variations exist in the functional properties of legume species (Table 2). In case of chickpea, the genotypes belonging to two distinct groups have shown large differences in certain functional properties as summarized in Table 3. The oil absorption of *kabuli* chickpea flours was 70-80% higher than those of the *desi* chickpea flour (Iyer and Singh 1997). Further, this study reported that *kabuli* chickpea starch was more viscous than *desi* chickpea starch, whereas the swelling power of *desi* was higher than the *kabuli* starch irrespective of heating temperature. On the other hand, nitrogen solubility index (NSI) of *kabuli* chickpea flour was significantly higher than that of the *desi* flour (Table 3).

When the oil absorption of a popular fried product of different legume flours was examined, the oil absorption of the fried product of chickpea differed significantly among genotypes, but clear cut differences were not observed between *desi* and *kabuli* groups of chickpea genotypes (Singh and Seetha 1993). According to this study, the oil absorption of deep-fried product was the highest in lentil (38.5%) and the lowest in pigeonpea (25.1%), indicating large variations among the commonly consumed pulses in India. The observed large differences were attributed to variations in the flour particle size, starch and protein contents of these legumes. Remarkable variations in oil and water absorption of bean varieties have been observed and these were found to be due to the nature and type of proteins present in the samples (Sathe and Salunkhe 1981).

Generally, cowpea products were equal to or superior to soybean in oil absorption, especially the protein concentrates (Sosulski et al. 1987). Further, these workers reported that oil emulsification value of dehulled seed flour of cowpea was several times greater than that of the whole seed flour of the same genotype of cowpea. The foaming capacities of legume flours and protein concentrates have been compared with those of the egg albumen. The air-classified protein concentrates and dehulled seed flour of cowpea and soybean showed foaming capacities and stabilities parallel to egg albumen, indicating no large variation in this functional property of these two legumes (Sosulski et al. 1987).

The gelling ability of legume flour appears to be a function of the nature and type of protein and starch. The gel consistency expressed as gel spread showed no remarkable differences between mung bean and pigeonpea (Singh et al. 1989). According to these workers, the gel strength of mung bean starch was higher than that of the pigeonpea starch, whereas no large differences in gel consistency of these two legumes were observed. The lower gel strength has been attributed to its low iodine affinity and lower amylose content in legumes (Lii and Chang 1981).

Soy protein concentrates produced by different manufacturers showed NSI values ranging from 5 to 70% (Victor and Inglett 1974). This had indicated remarkable variations in the NSI values of soy proteins. At pH 4.0, about 15-17% of cowpea proteins were soluble and thus extractable (Prinyawiwatkul et al. 1997). Unlike cowpea, most legumes have protein solubility of about 10% or less at low pH values (Sefa-Dedah and Stanley 1979). Analysis of 40 genotypes, showed a large variation in nitrogen solubility of cowpea flour and this was reported to be due to cultivars and processing of samples (Prinyawiwatkul et al. 1997). These workers reported a solubility range of 17-40% of unheated cowpea proteins at their isoelectric points (PI). The lower solubility of proteins

at pH near their isoelectric point may be due to their composition. Aggregation of legume proteins, particularly of albumin and globulin also adversely affect the protein solubility and digestibility (Singh 1985). The minimum nitrogen solubility of cowpea flour was found at pH 4.5 and nitrogen solubility increased at more acid or alkaline pH values (Sosulski et al. 1987).

There were considerable differences in such functional properties as emulsion and foam capacities within and between legume species (Sosulski et al. 1976). When expressed on both sample basis and protein basis, the emulsifying capacity of soy flour was remarkably greater than that of the peanut flour (McWatter and Holmes 1979). These workers further reported that soy flour emulsions were consistently more viscous than emulsions prepared from similarly treated peanut flour. The emulsification capacity of two different groups of chickpea genotypes also showed noticeable variations (Table 3).

Factors that affect the functional properties

The plant proteins are highly related to functionality and food applications. In the process of preparing legume food products for human consumption, heat treatments are commonly employed for a dual purpose to inactivate antinutritional factors and to improve the palatability. Nature and type of proteins and how they are denatured during processing are the key factors that affect the functional properties. Denaturation of proteins may be defined in general terms as any modification of the secondary, tertiary or quartenary structure of the protein molecule that does not break covalent bond (Victor and Inglett 1974). A change in protein structure as a result of processing is usually associated with changes in physico-chemical and functional properties. The denaturation of protein is most commonly assessed by the nitrogen or protein solubility index.

The nitrogen solubility index (NSI) is primarily influenced by the pH of the extracting solvent. Almost all proteins are affected by hydrogen or hydroxyl ions. The effects of pH and ionic strength on the behaviour of legume proteins have been the subjects of numerous studies in the past. The low pH and low ionic strength favour dissociation of protein molecules and thus affect the functionality of plant proteins (Sosulski and Fleming 1977).

In most legumes, minimum solubility occurs near the isoelectric point. The NSI was found to be highly pH-dependent with minimum NSI at pH 4.0 and maximum at pH 10.0 for cowpea flours (Giami 1993). The nitrogen solubility of legume flours is generally the highest at alkaline pH values and the lowest at acidic pH values.

The solubility of most legume proteins is markedly and irreversibly reduced, when severe heat treatment is applied. Heat causes progressive insolubility of proteins in soybean meal (Victor and Inglett 1974). It was further reported that both heat and moisture caused denaturations of protein as a result of which considerable changes in the physico-chemical and functional properties occurred. Boiling of legumes for sometime before flour preparation causes protein denaturation and thus a drastic decrease in solubility is observed (Prinyawiwatkul et al. 1997). The nitrogen solubilities and emulsifying properties of heated soy and peanut flours were reduced and the reduction increased as the heating time was increased (McWatters and Holmes 1979). Multiple regression analyses confirmed that heating time was the primary determinant in reduction of functionality of both flours and flour-derived products of soybean and peanut. Heat processing reduced the NSI and emulsion capacity of peanut flour and peanut-fortified sorghum flour (Singh and Singh 1991). The blanching process reduced the NSI of mung bean flour (Rosario and Flores 1981) and this reduction was attributed to the denaturation of proteins. According to Narayana and Narsinga Rao (1982), the nitrogen solubility decreased in heat-processed winged bean flours at all the pHs studied and at pH 4.5, minimum nitrogen solubility of 17% was observed in heat-processed flour as compared to 23% in the raw flour. However, dry heat treatment at 140°C for 20 min of peanut kernel, significantly improved protein solubility, whereas heating at 160°C beyond 10 min period decreased the level of water-soluble proteins in peanut meal (Patil et al. 1993). These workers pointed out that the solubility of proteins was known to be affected by their molecular size, configuration and charge and dry heat treatments affect these characteristics, thereby changing their solubility properties.

The water absorption capacity of the heat-treated cowpea flour was significantly higher ($P<0.05$) than those of the raw, germinated or fermented samples (Giami 1993). Heat treatment has been reported to increase the water absorption capacity of mung bean flour (Rosario and Flores 1981), peanut flour (Singh and Singh 1991) and winged bean flour (Sathe et al. 1982).

Polar amino acid residues of proteins have an affinity for water molecules and differences in water absorption capacity of soybean proteins and winged bean proteins could be due to the polar amino acid contents of these legumes (NAS 1975). However, this theory was ruled out by Narayana and Narasinga Rao (1982), who reported that polar amino acids were not responsible for differences in water absorption capacities of these legumes. They further suggested that conformational features of the proteins could cause the difference in water absorption capacity. During heat treatment, major proteins are disassociated into sub-units that might have more water binding sites than the native or oligomeric proteins (Catsimpoolas et al. 1970). It has also been noted that water absorption of proteins can be improved by partial denaturation, disassociation, unfolding and unsolubilization (Cheftel et al. 1985).

Autoclaving reduced the fat absorption in the black and red varieties, while roasting process decreased it in the black variety of horsegram flour (Diwakar et al. 1996). According to this study, the water absorption capacity was similarly expressed by black and red varieties and autoclaving or roasting improved the water absorption capacity of horse gram. As a result of heat treatment, gelatinization of carbohydrates and swelling of crude fibre may also occur, leading to increased water absorption. Prinyawiwatkul et al (1997) reported that heat processed flour of cowpea had greater water retention than

did flour from control and soaked seeds and attributed that the increase in water absorption might have been due to changes in concentration and structural confirmation of proteins.

The emulsion capacity response has been reported to be very sensitive to heat treatment. The emulsion capacity of soy flour was remarkably reduced as a result of heat treatment (McWatters and Holmes 1979). According to this study, the emulsion capacity reductions were more gradual for peanut flour, almost a straight-line effect than for soybean. The dissociation and partial unfolding of globular proteins, which expose the hydrophobic sites of amino acids have been reported to increase the emulsifying properties of soybean by increasing surface activity and adsorption at the oil-water interface (Nir et al. 1994). Protein-water interactions occur at polar amino acid sites on protein molecules. Most proteins contain numerous polar side chains along their peptide backbone, making them hydrophilic and hence affect the solubility and emulsion properties (Okaka and Polter 1979). Heat processing markedly decreased the emulsification capacity of winged bean flour at all pHs studied (Narayana and Narasinga Rao 1982).

Foam stability was slightly reduced in heat processed horsegram flours (Diwakar et al. 1996). According to this study, foamability is assumed to be dependent on the configuration of the protein molecules. Flexible protein molecules give good foamability, but highly ordered globular molecules give low foamability because they cannot reduce the surface tension of the air-water interface (Graham and Philips 1976). Heat processing diminished the nitrogen solubility of proteins by denaturation and thus, also reduced their foam capacity (Yasumatsu et al. 1972). Interestingly, it was noticed that germination had increased the foam capacity of cowpea flour, but decreased the foam stability compared to the raw sample (Giami 1993). A similar effect of germination on foam capacity and stability of protein isolates from yellow pea, faba bean and lentil has already been reported (Hsu et al. 1982). The choice of appropriate drying techniques for grain legumes in tropical countries is expected to influence physical, chemical and functional properties. Abu et al (1999) reported that foaming and emulsion properties of cowpea were adversely affected by sun-drying, which lasted 5 hr and the situation was aggravated by prolonged storage period. The sun-drying conditions particularly could have caused dissociation of some heat-sensitive stabilising agents (phospholipids, glycolipids) with respect to emulsion property (Sathe and Salunkhe 1981) and changes in albumin and globulin concentrations with regard to foaming property (Deshpande et al. 1983).

The least gelation capacity of cowpea flours increased as a result of heat treatment and cowpea flour was found to be more hydrophilic than lipophilic, regardless of the processing treatment (Prinyawiwatkul et al. 1997). These workers reported that at least 15% (dry w/v) heated flour was required as compared to 10% control flour for gel formation. Generally, legumes contain high protein and starch contents and the gelation capacity of flours is influenced by a physical competition for water between protein gelation and starch gelatinization. Heat processing results in considerable changes in certain functional properties such as gelation capacity and gel firmness and this was possibly due to changes introduced in protein molecules (Singh and Singh 1991).

In some legumes, the bulk densities of flours are influenced by processing practices. Bulk densities of the germinated and fermented flours of cowpea were reduced by 70.6% and 35.3%, respectively (Giami 1993). Processing treatments increased the bulk density, viscosity, water and fat absorption capacities of cowpea flour in the model system as compared to flour from untreated raw cowpea (Thakur and Puttaraj 1995).

The storage conditions also affect the functional properties of grain legumes. Numerous investigations have shown that grain legumes, in general, undergo adverse physico-chemical changes during storage in tropical climates (temperature 25-40°C, relative humidity 72-86%). The nitrogen solubility during storage at 65% RH remakably decreased in cowpea flour (Sosulski et al 1987). Further, these workers observed that oven drying of cowpea products had an adverse effect on functionality and nutritive value, especially when stored at 79% RH.

Future researchable areas

Grain legumes show great diversity in terms of the origin and agro-climatic regions, where they are grown. Some efforts are needed to be directed towards evaluation of genetic varieties of grain legumes with wider adaptability for their functional properties and how these could be related to the overall quality of the end-product.

Even though the interest of researchers for functional properties of legume flours has increased in recent years, there are several areas, where more systematic efforts are needed for understanding the functional properties with respect to processing and utilization of legume flours for various food formulations. The functional properties of soybean and peanut flours have been the topics of numerous investigations in the past, whereas other legumes have not been extensively studied, particularly the pulses commonly grown and consumed in India.

The identification and improvement of specific functional properties of legume flours are essential for determining the potential uses of various legume flours. The gelatinization characteristics of cereal starches offer some distinct advantages in cakes and muffins. This aspect has not been fully examined in grain legumes, particularly in view of the fact that legume flours are good supplements to cereals in making value-added bakery products.

The globular proteins of legumes are greatly influenced by the processing practices, particularly the heat treatments and hence adversely affect the NSI, PDI and protein digestibilities of legumes. Much efforts are needed to understand the mechanisms involved at the molecular levels and how these changes could be prevented to enhance the protein digestibility. The globular nature of legume seed storage proteins is well known. Unlike cereals, the specific functional role of this type of protein in the development of suitable food

products has not been adequately examined in different legumes and this needs to be studied.

Like cereals, the legume grains contain amylose and amylopectin fractions as the principal starch components. An understanding of these starch components in affecting the functional properties and end-use quality is essential particularly that of the cooking behaviour of legumes and their acceptability and this must receive considerable attention in the future. Generally, the bread doughs are viscoelastic, the characteristic which is noticeably changed, when legume flours are mixed with wheat flour for bread making. Legume starch pastes are more viscous than those of cereal starches and have a higher resistance to swelling and rupture than do cereal starches. Efforts should be made to improve the paste viscosity of legume flours and some legumes with desirable paste viscosity could be identified and developed, so that these could be used in composite flours with cereals for making value-added products.

Proteins are important in maintaining moisture in certain food products, particularly the bakery products. It is essential to understand the role of functional behaviour of legume proteins for enhanced uses in such value-added products.

Effects of processing methods on the functional properties of legume flours have been the subject of several studies in the past. However, information on the functional properties of legume flours particularly of Indian pulse crops as influenced by simple domestic processing methods is needed in view of their increased utilization.

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RESEARCH PAPER

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Extraction and Characterisation of Peroxidases from Oil Palm (*Elaeis guineensis.* Jacq) Fruit and Leaf

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Peroxidases from oil palm fruit mesocarp (MPOD) and leaf (LPOD) were isolated and their kinetics were studied. The former had an optimum pH of 5.5, while the latter had a pH of 5.0 Both MPOD and LPOD had high pH stabilities in the pH range at 4-10, LPOD being more stable in the pH range of 8-10. Both exhibted high thermal stabilities as compared to other sources, LPOD being more stable than MPOD. Using guaiacol as substrate, Michaelis constant (Km) for MPOD was 2 mM and for LOPOD, it was 7.5 mM. MPOD and LPOD showed greater activity during the early stages of fruit and leaf development, respectively and as maturation proceeded, their acitivities declined. MPOD and LPOD activities of the two parental varieties of oil palm, 'dura' and 'pisifera' were also compared.

Keywords : Oil palm, Peroxidase, Mesocarp, Oil palm fruit, Leaf, Maturation.

Peroxidase (EC. 1.11.1.7), a ubiquitous class of proteins is part of large group of enzymes, collectively known as oxidoreductases (Vamos-Vigyazo 1981). Various physiological functions have been attributed to peroxidase in nature. In plants, it is involved in hormone balance (Schneider 1970), catalyzes the oxidation of indole-3-acetic acid (Thomas et al. 1981), participates in the later stages of lignification (Mader and Fussi 1982), mediates the degradation of chlorophylls (Huff 1982), is involved in defence against infection (Wakamatsu and Takahama 1993) and oxidizes fatty acids and phenols (Florence and Frederic 1997). Peroxidase activity has also been used as an index of various operations associated with the processing of vegetables and fruits due to its thermal stability. It has also been used as an index of blanching in corn processing (Gardner and Inglett 1969), as an indicator of chilling injury (Haard and Timbie 1973) and in the cold treatment of pear embryos (Tao and Kahn 1976).

Peroxidase in the recent past assumed commercial significance due to its potential use in diagnostics and in industry. They are widely used as enzyme-labelled reagents in diagnostics, as in 'ELISA' kits, enzyme immunoassay tests etc. Peroxidase has great potential for application in the area of waste water treatment also. Once activated by H_2O_2, it catalyzes the oxidation of a variety of toxic aromatic compounds including phenols, biphenols, anilines, benzidines and related hetero aromatic compounds (Dec and Bollag 1994; Karam and Nicell 1997). They are also reported to catalyse the degradation of synthetic dyes (Spadara and Renganathan 1994). Recently, peroxidase has been incorporated in detergents as a biological bleaching agent (Showell 1998).

Oil palm (*Elaeis guineensis.* Jacq) is the second largest source of vegetable oil in the world. Palm oil is derived from oil palm fruit, which is a sessile drupe with a fleshy mesocarp (source of palm oil), enclosing a hard endocarp (source of palm kernel oil). Oil palm fruit mesocarp is known to contain an extremely active lipase that initiates the destabilization process of the oil (Abigor et al. 1985; Mohankumar et al. 1990). Subsequent action of the lipoxygenase and peroxidase on the fatty acids promotes autooxidation and results in the deterioration of oil quality. Knowledge on the biochemistry and kinetics of these enzymes, therefore, are of great commercial significance. In this context, oil palm lipase was examined from this angle (Henderson and Osborne 1991). However, the other two enzymes have not been investigated.

The present study was aimed at gathering basic information on peroxidase from oil palm fruit and its pattern of distribution. For comparison, oil palm leaf was also investigated for biochemical properties and the kinetics of this enzyme.

Materials and Methods

Plant material : Oil palm (*Elaeis guineensis.* Jacq) of 'tenera' variety was chosen for the present study. Fruits and leaves were collected from the oil palm plantation at Central Plantation Crops Research Institute (CPCRI), Palode, Thiruvananthapuram, India. Fully ripened fruits and mature leaves were used for the experiments and the materials were thoroughly washed before use.

Preparation of acetone powder : Acetone powder of fruit mesocarp and leaf was prepared by following the method of Nason (1955). Exocarp of the fruit was removed using a knife and the mesocarp tissue was sliced into small pieces. Mesocarp (10 g) was homogenised using a mortar and pestle and then extracted with 200 mL acetone cooled to -20° C. Acetone extract was filtered and the extraction was repeated 5 times, until a colourless filtrate was obtained. Residue obtained after filtration was allowed to dry at room temperature for 4 h. Leaves, after the removal of midribs were cut into small pieces and acetone powder was prepared as described for mesocarp. The acetone powder thus obtained was stored in a desiccator at 4°C. For all the subsequent experiments, acetone powder was used.

Extraction of peroxidase : All operations were performed in the temperature range of 0-4° C. Acetone powder of the mesocarp (5 g) was suspended in 0.1 M phosphate buffer, pH 7.0 (1:20), stirred at 4° C for 1 h and filtered through 4 layers

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of cheesecloth. It was centrifuged at 8000 rpm at 4°C for 15 min and the clear supernatant was used for peroxidase (MPOD) assay. Leaf peroxidase (LPOD) was extracted from acetone powder using the same procedure as MPOD, except that the buffer used was 0.1 M acetate buffer, pH 4.0 in the ratio 1:10.

Asaay of peroxidase : MPOD activity was determined using an assay system consisting of 20 mM guaiacol (0.5 mL), 0.1 M acetate buffer, pH 5.5 (2.1 mL), 40 mM H_2O_2 (0.2 mL) and the enzyme extract (0.2 mL) with a final volume of 3 mL. Oxidation of guaiacol was measured by the increase in absorbance at 470 nm (Chance and Machly 1955). One unit of enzyme activity was defined as the amount of enzyme required to increase the absorbance by 0.1/min. For LPOD, the assay buffer was 0.1 M acetate buffer at pH 5.0. All the experimental conditions for MPOD and LPOD were the same, unless stated otherwise.

Effect of pH : The optimum pH for peroxidase activity was determined by using buffers of pH values 2, 3, 4, 5, 6, 7, 8, 9 and 10 and employing the above assay procedure (Buffers used were; for pH 2 and 3, glycine-HCl buffer; pH 4 and 5, acetate buffer; pH 6 and 7, phosphate buffer; pH 8, tris-HCl buffer and pH 9 and 10, glycine-NaOH buffer). Values represented the mean of duplicate experiments.

Effect of substrate concentration : Activity of peroxidase at varying concentrations of guaiacol (1, 2, 3, 4, 5, 6 and 7 mM) (for LPOD up to 10 mM) and hydrogen peroxide (1, 2, 3, 4, 5, 6 and 7 mM) were determined and Km and V_{max} were calculated from Lineweaver-Burk plot.

Thermal stability : Thermal stability experiments were performed by subjecting the enzyme extract to heating at 30, 40, 50, 60, 70, 80, 90 and 100 °C. The enzyme extract diluted 2- fold with the assay buffer (1 mL) was taken in separate test tubes and kept at respective temperatures for 30 min. From each tube, an aliquot of 0.2 mL was withdrawn at 5 min intervals and cooled by immersing in ice and was assayed immediately for residual peroxidase activity. The values represented the mean of two independent experiments.

pH stability : Enzyme extract (2 mL) in different tubes were adjusted to pH values 2, 3, 4, 5, 6, 7, 8, 9 and 10 by adding 1M HCl or 1M NaOH. Each sample was kept for 30 min at ambient temperature (30-32 °C). At the end of the incubation period, the pH was adjusted back to initial pH (5.0) and the residual enzyme activity was assayed as before. The values represented the mean of duplicate assays.

Change in peroxidase activity during fruit and leaf maturation : Fresh oil palm fruits were collected separately from 4 different plants of 'tenera' variety at 4, 8, 12, 16, 20 and 24 weeks after anthesis (WAA). Mesocarp tissue (5 g) after the removal of exocarp, was extracted separately with 0.1 M phosphate buffer, pH 7.0 (1:20) and centrifuged at 8, 000 rpm at 4 °C for 20 min. The fat pad obtained was removed and the clear solution below the fat pad was used for enzyme assay. The values represented the mean of 4 independent experiments.

Oil palm leaves were collected from the same plants selected for collecting fruits. Leaves at 15, 30, 45, 60 and 75 days of maturation were selected for the study. Leaves (5 g) were cut into small pieces and ground using 0.1 M acetate buffer, pH 4.0 containing 1 % polyvinylpyrrolidone (1:10). It was filtered through 2 layers of cheesecloth and centrifuged at 8,000 rpm at 4°C for 10 min. The clear supernatant obtained was assayed for peroxidase activity. The values represented the mean of 4 independent experiments.

Varietal difference studies : For this study, two parental varieties of *E. guineensis* Jacq. namely 'dura' and 'pisifera' were selected. Fresh fruits and leaves from 4 different plants of each variety were used for the study. Extraction of peroxidase was done as described earlier except that fresh mesocarp tissue and leaflets (5 g each) were used, instead of acetone powder. Peroxidase activity was assayed as described before. The values represented the mean of 4 independent experiments for each variety.

Estimation of protein : Protein content of the mesocarp extract was determined by the method of Lowry et al (1951) and that of leaf by Bradford's dye binding method (1976) using bovine serum albumin as standard.

Results and Discussion

Buffers (0.1 M) with varying pHs (3-8) were employed to maximise the extraction of peroxidases from acetone powder and the results are depicted in Fig. 1. Peak extraction efficiency for LPOD was observed at pH 4.0, whereas for MPOD, maximum extractability was in the alkaline range of pH 7-8. While the LPOD showed peak extraction in a narrow range, in the acidic range, MPOD exhibited gradual increase with increase in alkalinity. It was also observed that the extractability of leaf peroxidase was 4-fold higher than fruit peroxidase. Fig. 2 demonstrates pH optimum for LPOD and MPOD at pH 5 and 5.5, respectively, when guaiacol was used as the substrate.

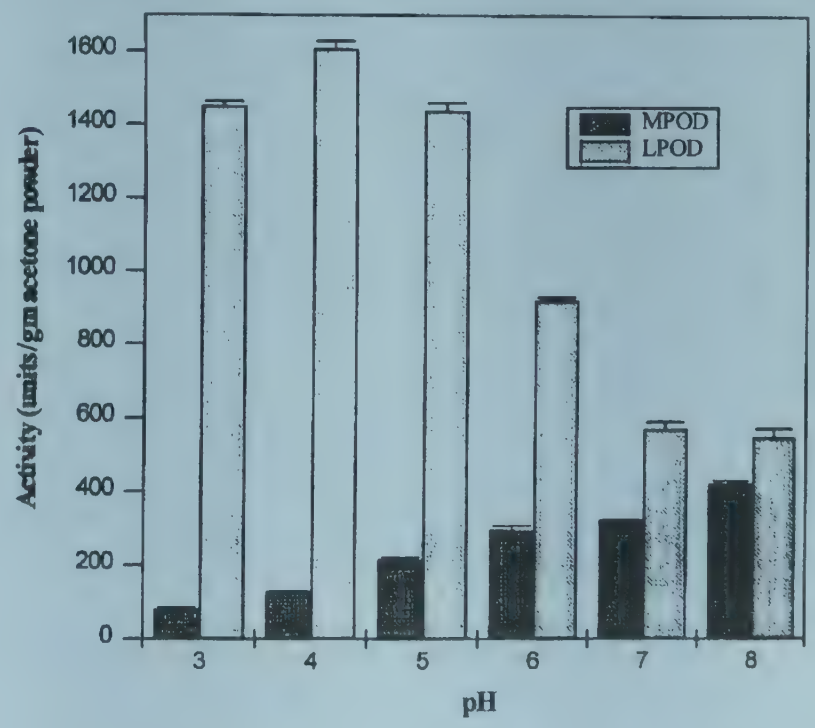

Fig. 1. Effect of pH on extractability of enzyme from acetone powder
Buffers used were : pH 3.0, glycine-HCl buffer; pH 4 and 5, acetate buffer, pH 6 and 7, phosphate buffer; pH 8; Tris-HCl buffer. Error bars represent the standard deviation of two independent experiments

A pH range of 40-5.5 suggested that the enzyme was more active in the acidic environment, such as in vacuoles (Takahama and Egashira 1994). In contrast to MPOD, which showed a sharp pH optimum, LPOD showed wide pH optima

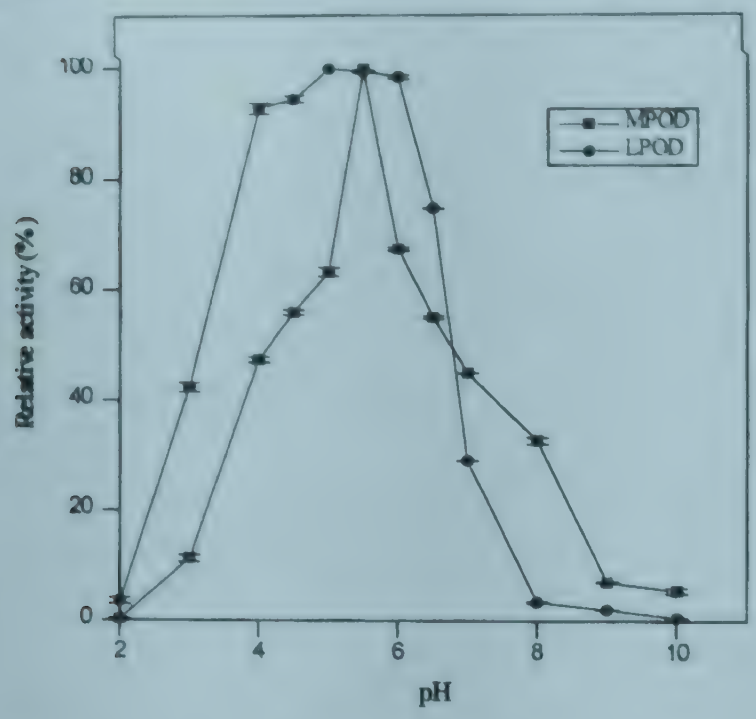

Fig. 2. Effect of pH on enzyme activity
Reaction system contained the assay buffer, 20 mM guaiacol,40mM H_2O_2 and the enzyme extract obtained from acetone powder. Values plotted on the graph represent the relative acitivity, i e., the ratio of the activity to the maximum activity (maximum activity for MPOD was 5.5 and for LPOD it was 5.0). Error bars represent the standard deviation of two independent experiments

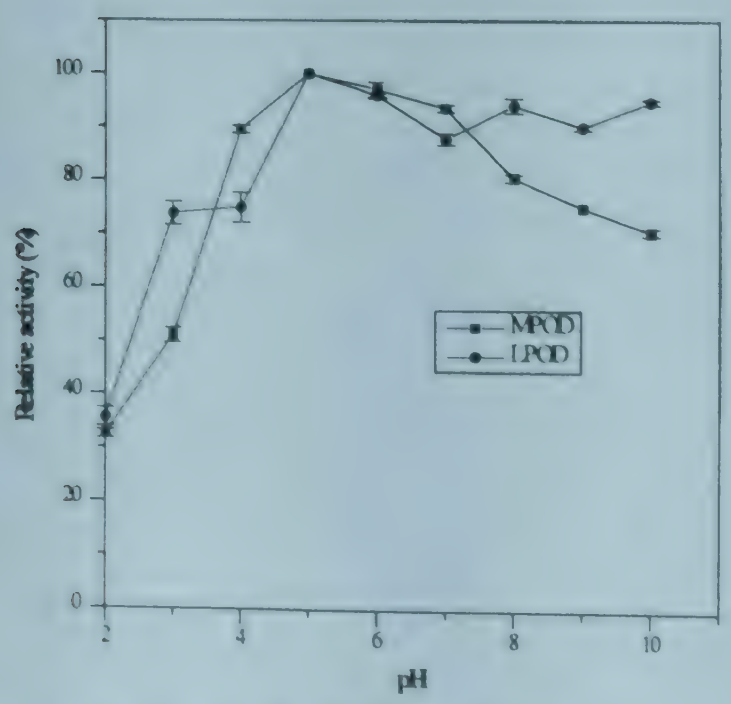

Fig. 3. Effect of pH on the stability of enzyme
An aliquot of the enzyme extract obtained from acetone powder was incubated at different pH values during 30 min at room temperature and the remaining enzyme activity was measured at the optimum pH (for MPOD 5.5 and for LPOD 5.0). Values plotted on the graph represent the relative activity i.e., the ratio of the activity to the maximum activity (for both) MPOD and LPOD, maximum activity was at 5.0. Error bars represent the standard deviation of two independent experiments

of 4-6, suggesting that the enzyme was active over a wide range of environmental conditions. This kind of a pH optimum may be due to the presence of isoenzymes, having various pH optima. Multiple pH optima were reported for peroxidase from various sources, most of them in the acidic range (Jen et al. 1980; Sessa and Anderson 1981).

Influence of pH on the stability of enzyme protein is shown in Fig. 3. MPOD and LPOD exhibited pH stability over a broad range, with greater stability in the pH range of 4 -10, while retaining more than 75% of activity. When compared to MPOD, LPOD was more stable in the pH range of 8-10. Peroxidase was reported to lose its activity at acidic pH due to the dissociation of heme moiety and hence, most of the reports showed the stability of peroxidase in the alkaline range (Pomar et al. 1997; Civello et al. 1995).

Fig. 4 illustrates the effect of temperature on the stability of MPOD. The loss of enzyme activity was marginal up to 60°C, but from 70°C onwards, thermal inactivation was faster. On incubating the enzyme at 60°C for 30 min, the loss of enzyme activity was only 20% retaining about 80% of its original activity. But at 70°C for 30 min, 50% of the enzyme activity was lost. In all the cases, enzyme was found to lose its activity at a much faster rate in the first 5 min, and subsequently, the reduction rate was slowed down. Thermal stability profile for LPOD is shown in Fig. 5. Though, the pattern of thermal inactivation rate of peroxydases from these two different sources seemed to be similar, LPOD appeared to be more resistant to heat. The inactivation curves for both were non-linear. Peroxidase has been reported to be one of the heat stable enzymes in plants. It has been reported that 6 min at 121°C is required to inactivate peroxidase in green

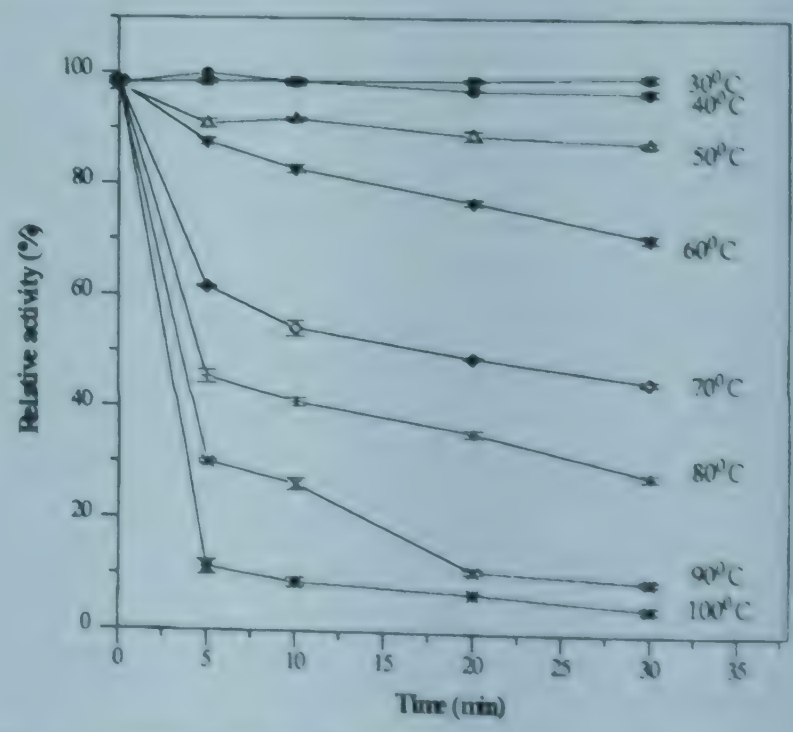

Fig. 4. Thermal stability of MPOD
Enzyme extract was incubated in acetate buffer, pH 5.5 for 30 min. Aliquots were taken out at 5 min intervals, immediately cooled in ice and assayed for peroxidase activity. Values plotted on the graph represent the relative activity, i.e., the ratio of the activity to the maximum activity. Error bars represent the standard deviation of two independent experiments

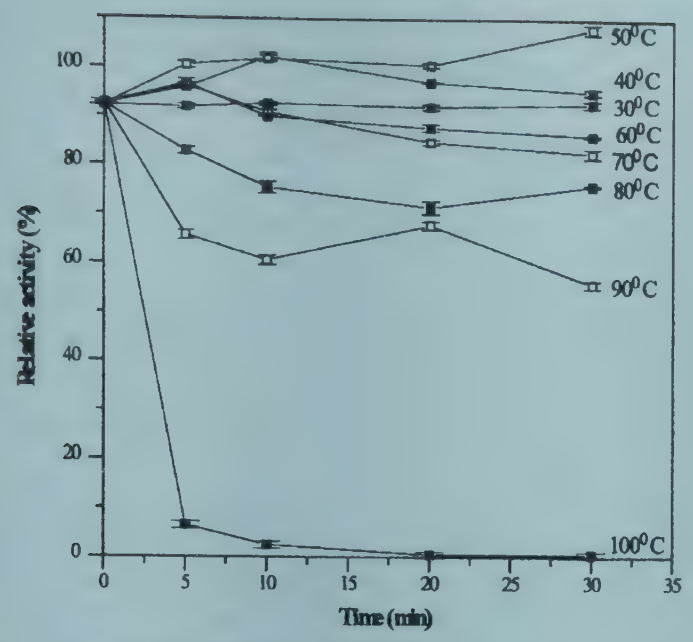

Fig. 5. Thermal stability of LPOD
Enzyme extract was incubated in acetate buffer, pH 5.0 for 30 min. Aliquots were taken out at 5 min intervals, immediately cooled in ice and assayed for peroxidase activity. Values plotted on the graph represent the relative activity, ie. the ratio of the activity to the maximum activity. Error bars represent the standard deviation of two independent experiments

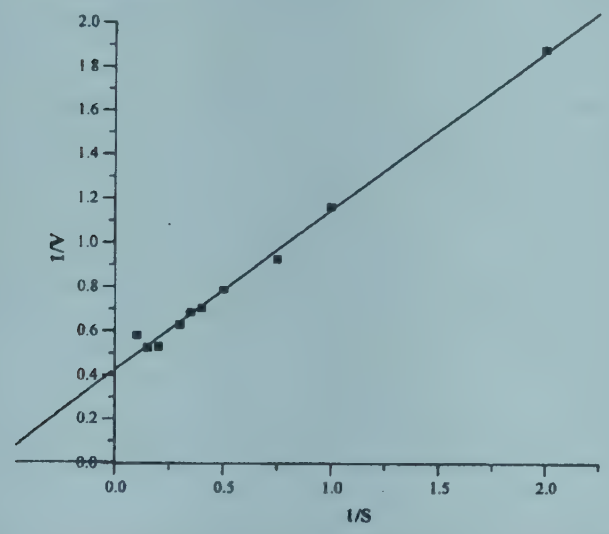

Fig. 6a. Lineweaver-Burk plot for guaiacol for crude MPOD
The reaction mixture contained 0.1M acetate buffer, pH 5.5; 40 mM H_2O_2, the enzyme extract and guaiacol at varying concentrations (1-7 mM)

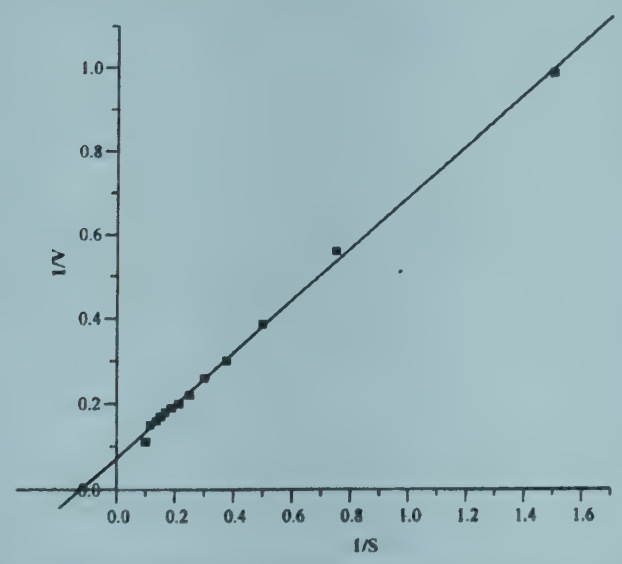

Fig. 6b. Lineweaver-Burk plot for guaiacol for crude LPOD
The reaction mixture contained 0.1M acetate buffer, pH 5.0; 40 mM H_2O_2, the enzyme extract and guaiacol at varying concentrations (1-10 mM)

peas (Halpin et al. 1989). Very high thermal stability of this kind may be due to factors like, the presence of large number of cystine residues in the polypetide chain (Mujer et al. 1983), formation of highly thermostable complexes from heat denatured enzyme protein (Vamos-Vigyzzo 1981) presence of sugars in the polypeptide chain etc. Not all the peroxidase reported are thermostable. Peroxidase from sources like strawberry (Civello et al. 1995), rice (Ito et al. 1991) etc. have low thermal stability, but coconut (Mujer et al. 1983) and cotton peroxidases (Triplett and Mellon 1992) exhibit high thermal stability.

The effect of different concentrations of guaiacol and hydrogen peroxide on the activity of MPOD and LPOD were also investigated. For MPOD, guaiacol and hydrogen peroxide

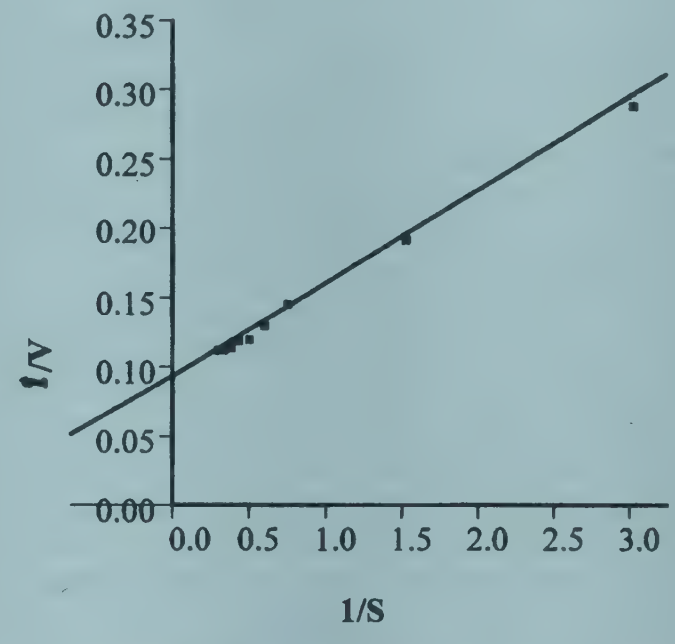

Fig. 7a. Lineweaver-Burk plot for hydrogen peroxide for crude MPOD
The reaction mixture contained 0.1M acetate buffer, pH 5.0; 20 mM H_2O_2, the enzyme extract and peroxide at varying concentrations (1-7 mM)

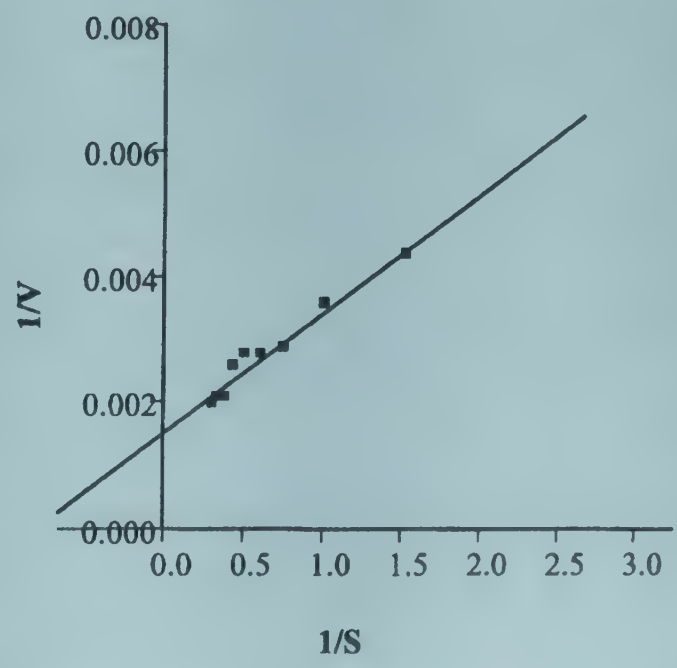

Fig. 7b. Lineweaver-Burk plot for hydrogen peroxide for crude LPOD
The reaction mixture contained 0.1M acetate buffer, pH 5.0; 20 mM H_2O_2, the enzyme extract and hydrogen peroxide at varying concentrations (1-7 mM)

concentrations ranging from 1-7 mM were used. From Lineweaver-Burk plot, it was observed that Km for guaiacol and hydrogen peroxide concentrations ranging from 1-10 mM were used. Km values for guaiacol and hydrogen peroxide were 7.5 mM and 1.45 mM, respectively for LPOD (Figs. 6 a, b and 7 a, b) MPOD exhibited greater affinity towards guaiacol than LPOD. But LPOD had greater affinity for hydrogen peroxide than MPOD. Using guaiacol as substrate, the Km for green peas was 10.2 mM (Halpin et al. 1989), for pea roots it was 5 mM (Fielding and Hall 1978), for soybean it was 5.9 mM (Sessa and Anderson 1981) and for tomato, 5-10 mM. (Jen et al. 1980). LPOD and MPOD exhibited high Km values for H_2O_2 than those reported earlier from other sources; pea roots, 0.3 mM (Fielding and Hall 1978) and soybean, 0.58 mM (Sessa and Anderson 1981). But for tomato peroxidase, it was 4 mM (Jen et al. 1980) and for green peas, it was 2.6-3.0 mM (Halpin et al. 1989). Peroxidase from *vicia faba* leaves has greater affinity towards guaiacol and H_2O_2 indicated by the very low Km for guaiacol and H_2O_2 as 0.62 mM and 0.83 mM, respectively (Takahama and Egashira 1991). Table 1 compares the activities of peroxidases in oil palm leaf and fruit. The total activity as well as the specific activity of leaf peroxidase was higher than fruit peroxidase.

To understand the activities of MPOD and LPOD as influenced by the age of the fruit and leaf samples, these specimens were collected periodically, viz., 4 weekly intervals for fruits and biweekly intervals for leaves. Fig. 8 illustrates the changes in the enzyme activity and specific activity of MPOD during fruit development. At 4 weeks after anthesis (WAA), peroxidase activity per gram mesocarp was almost 10-fold higher than that at 24 WAA (fully ripened fruit). As the fruit matured, there was a sharp reduction in the peroxidase activity up to 12 WAA, after which the rate of decrease was slowed down and it remained more or less static, until the fruit was fully ripened. In the case of LPOD, tender leaves exhibited greater enzyme activity per gram tissue and as the leaves matured, there was a sharp decline in enzyme activity. In the case of specific activity, it remained more or less same during maturation except that tender leaves showed slightly increased activity (Fig. 9). Both the activities remained more or less the same during subsequent developmental stages. Various reports are available in the literature regarding the changes in peroxidase activity during fruit ripening. In pear and tomato, peroxidase activity increased, while in blue berry peroxidase activity decreased as the fruit ripened (Frenkel 1972; Thomas et al. 1981). In banana, no change in the activity of soluble peroxidase was observed during ripening (Haard and Tobin 1971). Strawberry fruit peroxidase exhibited very high specific activity during the initial stages of ripening, followed by a sharp decline in fully ripened fruits (Civello et al. 1995).

TABLE 1. OIL PALM FRUIT AND LEAF PEROXIDASES: A COMPARISON

Crude extract	Total activity, units	Total proteins, mg	Specific activity, units/mg protein
MPOD	830	13.80	60.18
LPOD	20325	34.74	585.06

25 g each of the mesocarp acetone powder and leaf acetone powder were used the extraction of fruit and leaf peroxidase, respectively.

Activities of MPOD and LPOD for the two parental varieties viz., 'dura' and 'pisifera' are presented in the form of a histogram (Fig. 10). Within the same variety, leaf tissue

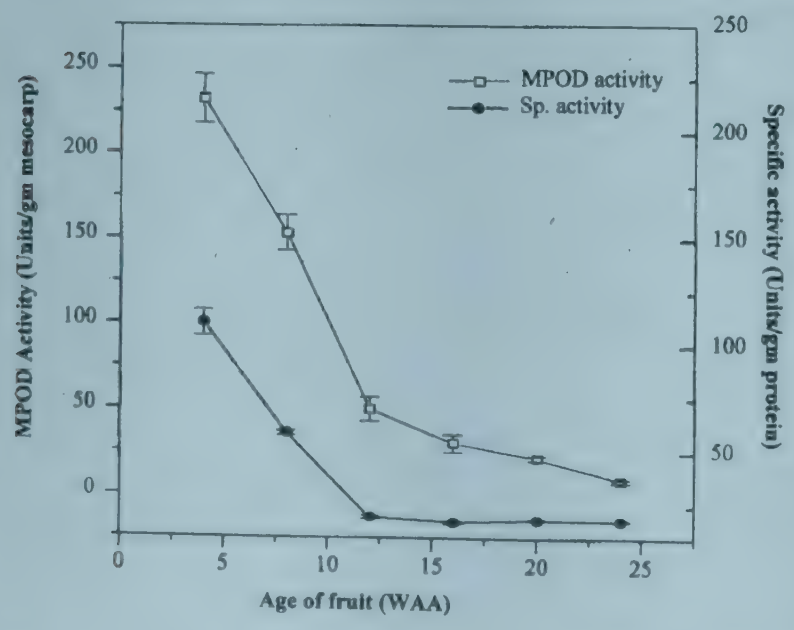

Fig. 8. Changes in the enzyme activity and specific activity of peroxidase during fruit development
Enzyme extracts were obtained from fresh fruit mesocarp which were collected from oil palm 4, 8, 12, 16, 20 and 24 weeks after anthesis. Mesocarp tissue was cut into small pieces, ground well and extracted with 0.1 M phosphate buffer, pH 7.0 and centrifuged. The clear supernatant obtained after centrifugation was assayed for peroxidase activity. Error bars represent the standard deviation of four independent experiments

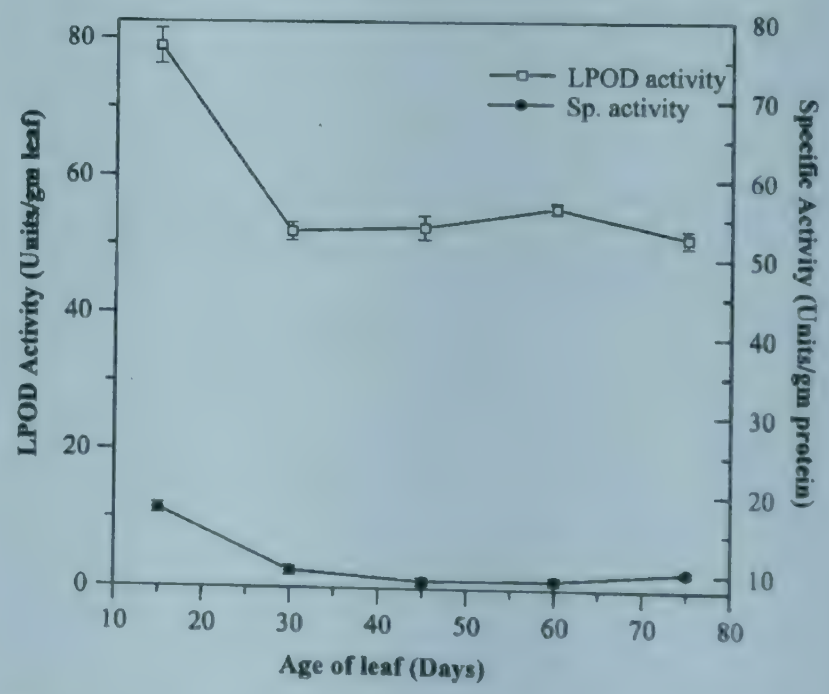

Fig. 9. Changes in the enzyme activity and specific activity of peroxidase during leaf maturation
Enzyme extracts were obtained from fresh leaves which were collected from oil palm at an interval of 15 days. Leaves were cut into small pieces, ground well and extracted with 0.1 M acetate buffer, pH 4.0. The clear supernatant obtained after centrifugation was assayed for peroxidase activity. Error bars represent the standard deviation of four independent experiments

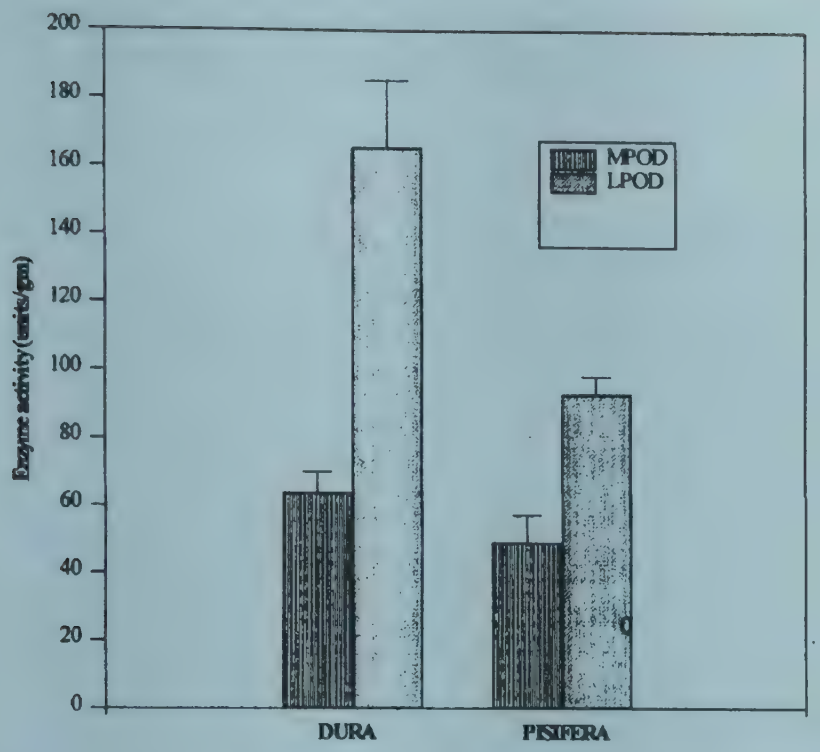

Fig. 10. Peroxidase activity of two parental oil palm varieties The enzyme extracts were prepared from the fruit mesocarp and fresh leaves of the 'dura' and 'pisifera' varieties. The error bars represent the standard deviation of four independent experiments.

was found to contain 2 to 3 times greater peroxidase activity, when compared to that of the respective fruit tissue. Between the varieties, the 'dura' leaf appeared to posses, significantly higher level of LPOD activity as compared to that of 'pisifera'. However, fruits from these varieties had comparable MPOD activities on per gram tissue basis. Per gram mescocarp and leaf, 'dura' has greater peroxidase activity than 'pisifera'. But in both the cases, their specific activities remained almost same. More in-depth study may be required on this approach to exploit this genetic diversity of peroxidase to identify oil palm variety at the seedling stage so that low yielding 'dura' could be eliminated from commercial planting.

Peroxidase is a commercially important enzyme, as an ingredient of detergents and in the treatment of effluents for dye decolourisation. By virtue of its thermal and pH stability and oil palm leaf being a rich source of this enzyme, LPOD need to be studied from these perspectives.

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RESEARCH PAPER

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A Study on the Effect of Different "Hurdles" on the Rheological Properties of Fried *Paneer* by Response Surface Methodology

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The effect of different 'hurdles' viz., water activity (a_w), pH and heat treatment (F) on the rheological properties viz., hardness and chewiness of fried *paneer* immediately after processing as well as during storage was studied by response surface methodology (Hoke's design). The different levels studied were : a_w 0.90, 0.94, 0.98; pH 4.7, 5.3, 5.9; F-value 0.2, 0.5, 0.8 and potassium sorbate 0.0, 0.05, 0.10 %. These parameters profoundly influenced the hardness and chewiness during processing as well as during storage. The data were fitted to second order polynomial regression equation. Constants for the best fit polynomial equation for the change in rheological characteristics, linear-linear form in case of effect of processing and ln-linear form in case of storage changes in chewiness have been given. Using these equations, the changes in hardness and chewiness as a function of the above parameters have been predicted and displayed in the form of response surfaces. Decrease in a_w and pH increased hardness and chewiness, whereas heat treatment decreased them. Based on the predicted response surfaces, the minimal changes occurred in hardness at the following values : a_w 0.90-0.93 at pH 5.1-5.9; a_w 0.918-0.948 at F-value 0.37-0.79. Minimal changes in chewiness occurred at a_w 0.90-0.97 at pH 4.7-5.3; a_w 0.900-0.945 at F-value 0.20-0.35. Synergistic effect of these parameters was also observed. These observations will be of significance in canning of *paneer* and *paneer*-based products.

Keywords : *Paneer*, Hardness, Chewiness, Response surface methodology, Hurdles.

Hurdle technology, also known as combined methods of preservation is gaining importance world over in recent years, as at involves judicious use of combined effects of bacteriostatic/bactericidal parameters (termed as "hurdles") to increase the shelf life of foods, (Leistner 1999). There are many reports on the combined effects of "hurdles" on the sensory and microbiological qualities of foods, but studies on hurdle effects on rheological characteristics are scarce. Rheological properties of *paneer* are mainly dependent on the processing parameters like coagulation temperature, pH of coagulation, intensity, time of pressing etc.(Sachdeva 1983). However, water activity (A_w or a_w) and pH of *paneer* and the heat treatment it receives during curry making also play equally important role in effecting the rheological properties, which have a bearing on the eating qualities. Food being a complex system, changes taking place are the result of individual and combined (synergistic) effects of several parameters, belonging either to processing or storage. Practically it is very difficult to study and quantify individual and combined effects separately; however, a suitable statistical tool can be employed for the purpose. Response Surface Methodology (RSM) is an ideal method to study and quantify the individual and combined effect of different parameters. This method has been successfully used in product development (Ylimaki et al. 1988) and modelling microbial growth (Thayer et al. 1987). Henika (1972) described response surface analysis as a useful statistical tool for analysing experimental data to optimise the physical properties of the food products using different levels of ingredients. Since then, the response surface analysis has been used in several areas like studies of protein denaturation (Nielsen et al. 1973), bacterial growth (Schroder and Busta 1973), high protein bread (Henselman et al. 1974), power consumption of dough sheeting (Raghavan et al. 1996), heat treatment optimisation of dehulling pigeon pea (Phirke et al. 1999) and enzyme-aided extraction of soybean (Kashyap et al. 1997). Reports on effect of these parameters, more so their combined effects on rheological parameters of foods, in general and dairy products, in particular are scarce.

In this investigation, RSM was used to study as well as quantify the effects of water activity, pH and heat treatment on the hardness and chewiness of fried *paneer.*

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Materials and Methods

Fried paneer : Fresh *paneer* was procured from the Institute's Experimental Dairy, cut into 2 cm cubes, fried in refined oil at 175-185°C till golden yellow colour. The fried *paneer* had the following composition: moisture 29.7 %, fat 38 % and proteins 25.6 %.

Experimental design : Hoke's response surface design (Thompson 1982) consisting of 4 variables was followed (Table 1).

Variables : The fried *paneer* cubes were subjected to heat treatment at different water activity, pH and potassium sorbate levels in tin cans. These levels were decided as per Hoke's response surface design, which consisted of 4 variables (viz., A_w, pH, F-value and potassium sorbate) with three levels in each variable. Different levels are shown in Table 2.

Adjustment of pH and a_w : Each can was envisaged to contain 40 g fried *paneer* cubes and 160 g of humectant (glycerol)-acidulant (citric acid) solution. To calculare the quantity of citric acid required to be added to a can to adjust the pH of *paneer* to different levels, the following procedure was followed: Fried *paneer* cubes (40 g) were ground in a pestle and mortar by adding 160 ml of distilled water in instalments. The mixture was transferred to a 250 ml beaker and its pH brought down by adding 25% citric acid solution

TABLE 1. THE HOKE'S RESPONSE SURFACE DESIGN FOR THE FOUR INDEPENDENT VARIABLES

'Hurdle' Combination No.	Variables: Water activity, X_1	pH, X_2	F-value, X_3	Potassium sorbate, X_4
A	-1	0	0	0
B	0	-1	0	0
C	0	0	-1	0
D	0	0	0	-1
E	-1	-1	-1	-1
F	-1	1	1	1
G	1	-1	1	1
H	1	1	-1	1
I	1	1	1	-1
J	1	1	-1	-1
K	1	-1	1	-1
L	1	-1	-1	1
M	-1	1	1	-1
N	-1	1	-1	1
O	-1	-1	1	1
P	0	1	1	1
Q	1	0	1	1
R	1	1	0	1
S	1	1	1	0

+ 1 = highest limit of variable taken for study; 0 = Mid point; -1 = lowest limit of variable taken for study

TABLE 2. LEVELS OF 'HURDLES' USED FOR THE HOKE'S EXPERIMENTAL DESIGN

Hurdles	Symbol	Hurdle levels tested: -1	0	+1
Water activity a_w	X_1	0.90	0.94	0.98
pH	X_2	4.70	5.30	5.90
F-value	X_3	0.20	0.50	0.80
Potassium sorbate, %	X4	0.00	0.05	0.10

TABLE 3. INGREDIENTS REQUIRED PER CAN (200 G) TO ATTAIN DIFFERENT LEVELS OF "HURDLES"

Fried *paneer*, g	Glycerol, g (water, g) for a_w			Citric acid, mg for pH			Potassium sorbate, mg for levels		
	0.98	0.94	0.90	5.9	5.3	4.7	0.0%	0.05%	0.1%
40	16 (144)	42 (118)	62 (98)	0	200	375	0	100	200

to 4.7. The quantity of citric acid required to bring down the pH to 5.3 and 4.7 was recorded. The values are shown in Table 3.

For adjusting a_w, the quantities of glycerol and distilled water to be taken (Table 3) in cans to get the a_w value of 0.90, 0.94 and 0.98 were worked out using Raoult's law. For this purpose, moisture present in 40 g of fried *paneer* was also taken into consideration.

$$\text{Raoult's law :} \quad A_w = \frac{N_1}{N_1 + N_2}$$

where, N_1 = no. of moles of solute

N_2 = no. of moles of water

The can contents were ground in a pestle and mortar and the a_w was checked by Rotronic Hygroskop DT, Switzerland.

Packaging and sealing : Forty g of fried *paneer* cubes were weighed into cans and required quantities of glycerol, water, citric and potassium sorbate corresponding to desired a_w and pH values were added. In such manner, tin cans containing fried *paneer* cubes with 19 different combinations of a_w, pH, F-value and potassium sorbate as per Hoke's response surface design (Table 1) were prepared. The cans were sealed and kept at about 5°C for equilibration for about 10 h (overnight). The sealed cans were then heat-treated at corresponding F-values.

Processing time for desired F-value : Processing times for getting required F-values were determined by integrating heating and cooling curve data obtained experimentally by measuring the temperature of *paneer* cubes inside a can during heating and cooling. The method described by Stumbo (1973) was adopted.

F-value	Operator's processing time, min
0.2	9.6
0.5	15.6
0.8	21.6

Rheological measurements : After processing, the cans were kept at 30°C for about 2 months and at appropriate intervals, the rheological characteristics (viz. hardness and chewiness) were determined using Instron universal testing machine (sample size 2.0 cm, compression 80%, load cell 1000 N, speed of chart 250 mm/min, double cycle) by the procedure of Brady et al (1985). Duplicate readings were made.

Statistical analysis : The data of all rheological characteristics immediately after processing and during storage were tabulated. The reaction rate constants (k-values) for changes in rheological characteristics during storage were computed by fitting the data to different kinetic order equations (Singh 1991).

In order to estimate a_w, pH and F-value effects on each objective response i.e., hardness/chewiness/their k-value, the data were fitted to the polynomials of second order, using multiple regression procedure by MSTAT software. The generalised polynomial form is given below :

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{11}X_1^2 + b_{22}X_3^2 + b_{33}X_3^2 + b_{44}X_4^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4$$

where,

Y = hardness or chewiness (dependent variable); X_1 = water activity (independent variable); X_2 = pH (independent variable); X_3 = F-value (independent variable); X_4 = Potassium sorbate, % (independent variable)

TABLE 4. EXPERIMENTAL DATA OF RHEOLOGICAL PARAMETERS OF FRIED *PANEER* PROCESSED AND STORED AT VARIOUS "HURDLE" COMBINATIONS

Code	Hurdle' combination A_w	pH	F	Potassium sorbate	Hardness, mN	Chewiness, mN. mm	k-hardness, day^{-1}	k-chewiness, day^{-1}
A	0.90	5.9	0.8	0.10	957.5	1393.5	-0.0086	-0.0037
B	0.90	5.9	0.8	0.00	677.5	1405.2	-0.0055	-0.0025
C	0.90	4.7	0.8	0.10	1015.0	1692.5	-0.0086	0.0025
D	0.94	5.9	0.8	0.10	942.5	2073.7	-0.0249	-0.0276
E	0.98	4.7	0.8	0.10	245.0	306.2	-0.0174	-0.0015
F	0.98	4.7	0.8	0.00	265.0	224.2	0.0082	0.0110
G	0.98	5.3	0.8	0.10	110.0	117.6	-0.0030	-0.0043
H	0.98	5.9	0.8	0.05	95.5	115.0	0.0064	0.0016
I	0.98	5.9	0.8	0.00	114.0	165.7	0.0063	0.0047
J	0.90	5.3	0.5	0.05	705.0	1413.3	-0.0039	-0.0073
K	0.94	4.7	0.5	0.05	795.0	2289.2	0.0076	0.0001
L	0.94	5.3	0.5	0.00	267.5	620.1	0.0054	0.0025
M	0.98	5.9	0.5	0.10	134.8	339.1	-0.0018	-0.0157
N	0.90	4.7	0.2	0.00	1360.0	1061.7	0.0032	0.0192
O	0.90	5.9	0.2	0.10	650.0	966.9	-0.0085	0.0055
P	0.94	5.3	0.2	0.05	313.3	355.1	-0.0071	0.0048
Q	0.98	5.9	0.2	0.10	182.0	278.2	-0.0086	-0.0094
R	0.98	5.9	0.2	0.00	266.0	566.7	-0.0147	-0.0351
S	0.98	4.7	0.2	0.00	270.7	404.2	0.0060	0.0079

Perspective response surfaces were produced from the equations by holding one variable at fixed value and changing the other variables.

Results and Discussion

Hardness and chewiness are important rheological parameters for *paneer.* These were found to be profoundly dependent on a_w, pH and heat treatment given and changed with changes in other parameters as evident from the data given in Table 4. The data were subjected to the second order multiple regression equation in four forms. R^2 values presented in Table 5 show that the following forms of the equation are the best fit: Effect of processing on hardness: linear-linear; chewiness: ln-linear; rate of change in hardness and chewiness during storage: linear-linear. The constants for the respective equations are given in Table 6.

Perspective response surfaces (3-dimensional plots) were plotted using hardness and chewiness values in a_w range of 0.90-0.97, pH range of 4.5-5.9 and F-value range of 0.1-0.8. Predicted values were obtained by keeping one factor constant and changing the other two factors at potassium sorbate level of 0.0 %. Potassium sorbate was included as one of the variables in the present study, because it is one of the common preservatives used in processed foods. However, its effect on the rheological characteristics of *paneer* is not discussed in this paper and hence the response surfaces were obtained by keeping the levels of potassium sorbate at 0.

Hardness increased with decrease in a_w. At a_w 0.97, the hardness was 152.7 mN and at a_w 0.90, it was 772.4 mN (at pH 5.9 and F 0.5). This increase was almost linear. The trend was similar at all pH levels. With change in pH, the hardness first decreased and then increased. Hardness at pH 5.9 was 152.7 mN, at pH 5.3, it was 65.2 mN and at pH 4.7, it was

TABLE 5. R^2 VALUES OF DIFFERENT EQUATIONS FITTED TO THE RHEOLOGICAL DATA OF FRIED *PANEER*

Parameters	Equation form: Linear-Linear	In-Linear	Linear-In	In-In
Hardness	0.84	0.70	0.63	0.52
k-hardness	0.91	0.82	0.75	0.41
Chewiness	072	0.79	0.63	0.52
k-chewiness	0.97	0.80	0.82	0.65

TABLE 6. REGRESSION EQUATION COEFFICIENTS AND R^2 VALUES FOR HARDNESS AND CHEWINESS CHANGES IN FRIED *PANEER*

Partial' co-efficient	Response: Hardness	k-hardness	Chewiness	k-chewiness
b_0	-5504.37	-0.0878	425.04	0.5634
b_1	70185.09	0.5602	-738.32	-0.5097
b_2	-8034.31	-0.0673	-23.75	-0.0830
b_3	-82.67	-0.0517	16.13	-0.2165
b_4	-46036.38	0.4903	-43.63	-0.6196
b_{11}	-49265.98	-0.3279	365.2	0.0366
b_{22}	548.37	0.0041	1.69	0.0019
b_{33}	845.89	-0.0154	-7.91	0.0462
b_{44}	-868.93	-0.3487	-37.25	0.894
b_{12}	2210.02	0.0176	5.91	0.0574
b_{13}	100.77	0.0467	-9.24	0.1650
b_{14}	23017.06	-0.6088	18.34	0.1157
b_{23}	-210.65	0.0055	-0.14	0.00
b_{24}	3537.08	0.0248	3.12	0.00
b_{34}	8862.05	-0.0774	21.34	0.0027
R^2	0.84	0.91	0.79	0.97
Equation significance	**	**	*	**

** Significant at P<0.01, * significant at P<0.05

372.5 mN (at a_w 0.97) (Fig.1). Similarly, the heat treatment given to *paneer* too had a significant effect on hardness. *Paneer* cubes became slightly softer with increasing intensity of heat treatment. At 0.2 F, the hardness was 343.4 mN and at 0.8 F, the hardness was 114.2 mN (at pH 5.9 and a_w 0.97) (Fig. 2). The perspective response surfaces, which are almost uniform indicate little interaction effect of a_w-pH or a_w-heat treatment (Fig. 1 and 2).

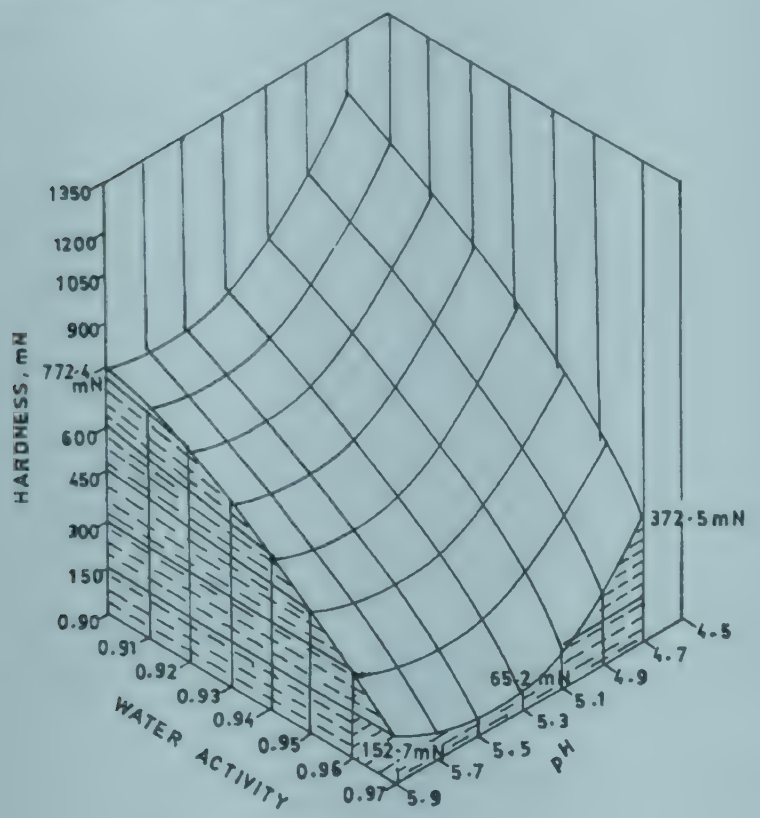

Fig. 1. Response surface of change in hardness of fried *paneer* as influenced by different levels of water activity and pH (fixed variable, F-value = 0.5)

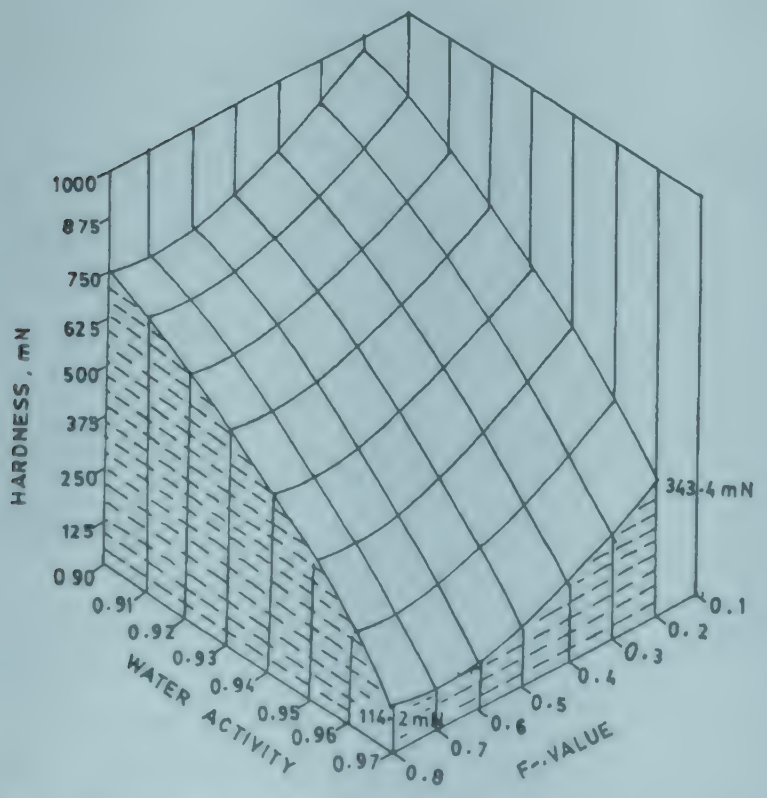

Fig. 2. Response surface of change in hardness of fried *paneer* as influenced by different levels of water activity and F-values (fixed variable, pH = 5.9)

Fried *paneer* was very chewy at low a_w and low pH values. Chewiness increased with decreasing a_w and pH. At pH 4.7, the chewiness was 617.6 mN. mm at an a_w 0.97. When a_w decreased to 0.90, the chewiness enhanced to 6037 mN.mm, showing a 10 times increase. A tapering top corner of the response surface (Fig. 3) indicates a_w-pH interaction effect. It means that heating of fried *paneer* at low pH and a_w levels markedly increases chewiness. At a given pH and a_w, increased intensity of heat treatment slightly increased the chewiness initially, but after 0.4 F-value, the chewiness decreased. The increasing curvature of the response surface (Fig. 4) indicates a_w-F value interaction.

No reports exist so far as to how a_w affects hardness of *paneer*. However, hardness of raw *paneer* (a_w : 0.994) is reported to be 12.89 mN (Roy 1990) and that of *paneer* with a_w 0.95 was reported to be 688 mN (Jayaraj Rao and Patil 1999). Chewiness of raw *paneer* was .58.14 mN.mm (Roy 1990) and that of *paneer* with a_w 0.95 was 1403.5 mN.mm (Jayaraj Rao and Patil 1999).

Changes during storage : The changes in hardness and chewiness during storage of fried *paneer* at 30°C are expressed as reaction rate constants (k-values). These changes were found to follow zero order kinetics. The magnitude of k-value indicates rate of change: the positive sign indicates increase and the negative sign indicates decrease in the parameter. The hardness decreased during the storage, when fried *paneer* was processed at high pH and low a_w levels and the rate of decrease was highest at low a_w. Otherwise, the hardness of *paneer* increased with progress in storage period and the rate of increase was faster at high a_w and low pH levels (Fig. 5). The rate of change was faster with decreasing a_w and decreasing pH. The a_w-pH interaction effect was slightly evident as the response surface was slightly depressed at low a_w and

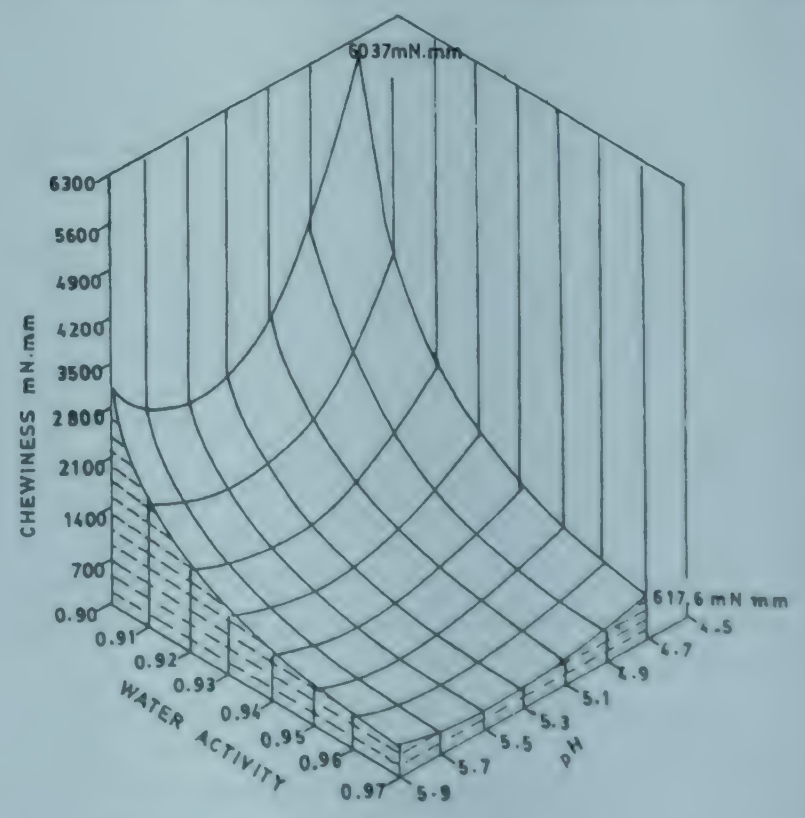

Fig. 3. Response surface of change in chewiness of fried *paneer* as influenced by different levels of water activity and pH (fixed variable, F-value = 0.5)

high pH region. A zero reaction rate zone was identified at pH 5.1-5.9 and a_w 0.90-0.93, as shown by intersecting the response surface with a zero-reaction rate plane (Fig. 5). Low intensity of heating seemed to induce more rapid changes during storage, indicating that changes in hardness were not only due to effect of heat *per se*, but also due to continuing internal changes, possibly structural. The zero reaction rate zone as identified by intersecting the response surface with zero reaction plane was F-value 0.37-0.79 and a_w 0.918-0.948 (Fig. 6). The chewiness either decreased or increased during storage depending on a_w and pH. The rate of change was maximum at high pH-high a_w and low pH-low a_w levels. Synergistic effect of a_w- pH was evident by the extended top corner of the response surface. The zero reaction rate zone was identified at a_w 0.90-0.97 and pH 4.7-5.3 (at 0.5 F) (Fig. 7). When fried *paneer* was processed at different values at a given a_w, the rate of change increased with increasing heat intensity. At low a_w, the rate of change was maximum. At 0.2-0.3 F and a_w 0.90-0.955, the chewiness increased during storage as indicated by + sign of k values. The zero reaction rate zone identified was a_w 0.90-0.945 and F- value 0.2-0.35 (Fig. 8). It may be commonly observed that the fried *paneer* in stored canned *paneer* curries sold in market is hard, rubbery and chewy. It was observed that canning of fried *paneer* at 15.0 F-value increased the chewiness about 4 times (Jayaraj Rao and Patil 1999).

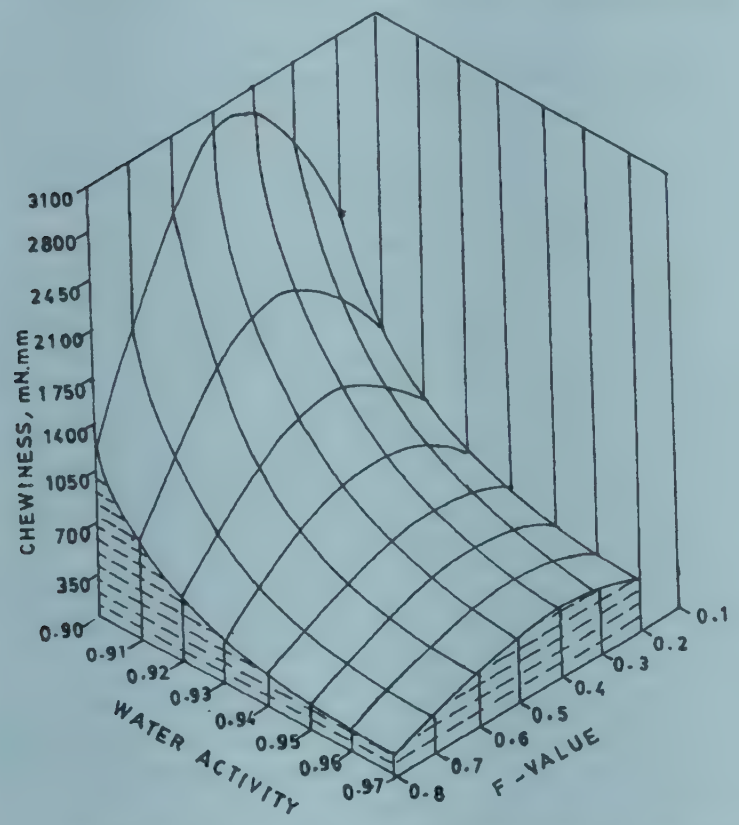

Fig. 4. Response surface of change in chewiness of fried *paneer* as influenced by different levels of water activity and F-value (fixed variable, pH = 5.9)

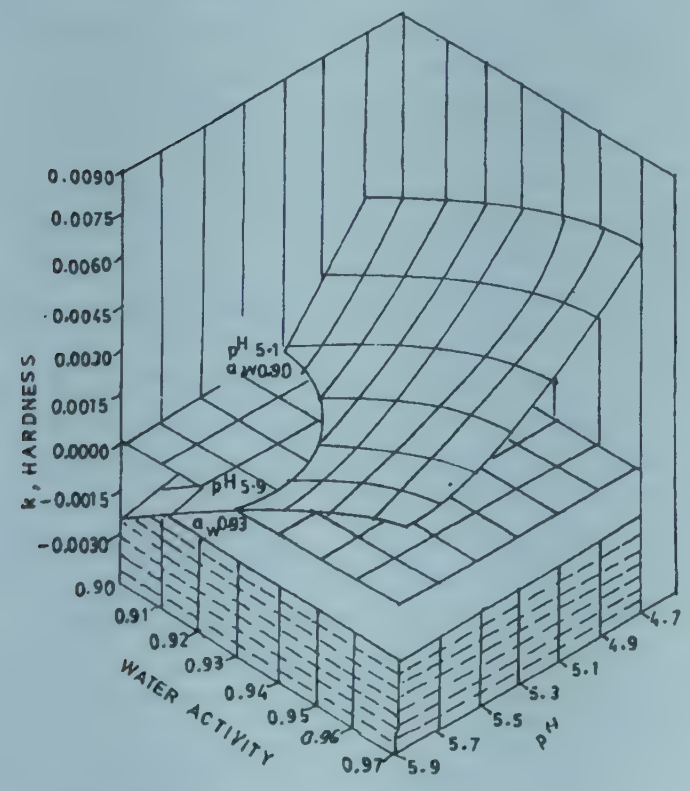

Fig. 5. Response surface, intersected by the plane of zero reaction rate, for change in hardness reaction rate during storage of fried *paneer* (fixed variable, F-value = 0.5)

Milk proteins, which have been denatured, respond to physical factors in several ways. At a pH of about 5.3 and a temperature of 75°C, calcium para-caseinate becomes flexible and elastic (Kosikowski 1978), and the acid coagulated milk protein particles, when processed hot mat with each other as in *paneer* making. However, at low temperatures (less than 50°C) the matting is very difficult. The dry casein particles are free flowing and on rehydration, they absorb 30-43 g water/ 100 g (Kinsella and Fox 1987). During heating and coagulation, it can be expected that the proteins undergo several changes commonly associated with any protein unfolding, breakage of bonds, structural rearrangement etc. On reduction of a_w, the *paneer* loses water and becomes hard possibly because of shrinkage caused by the blockage of pores and at low pH, *paneer* becomes brittle. On frying, the proteins in *paneer* undergo extensive denaturation. This denaturation is further aggravated by heat treatment in terms of structural

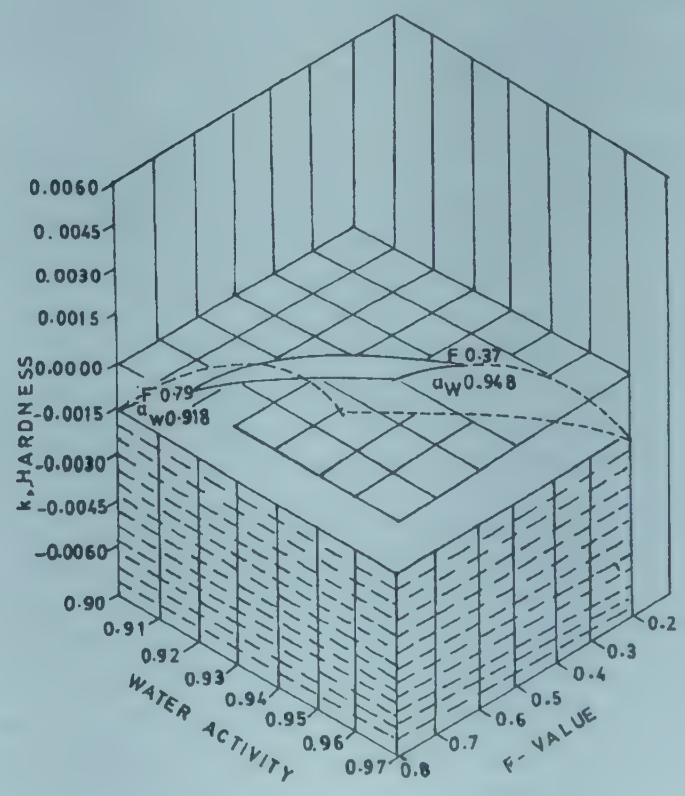

Fig. 6. Response surface, intersected by the plane of zero reaction rate, for change in hardness reaction rate during storage of fried *paneer* (fixed variable, pH = 5.9)

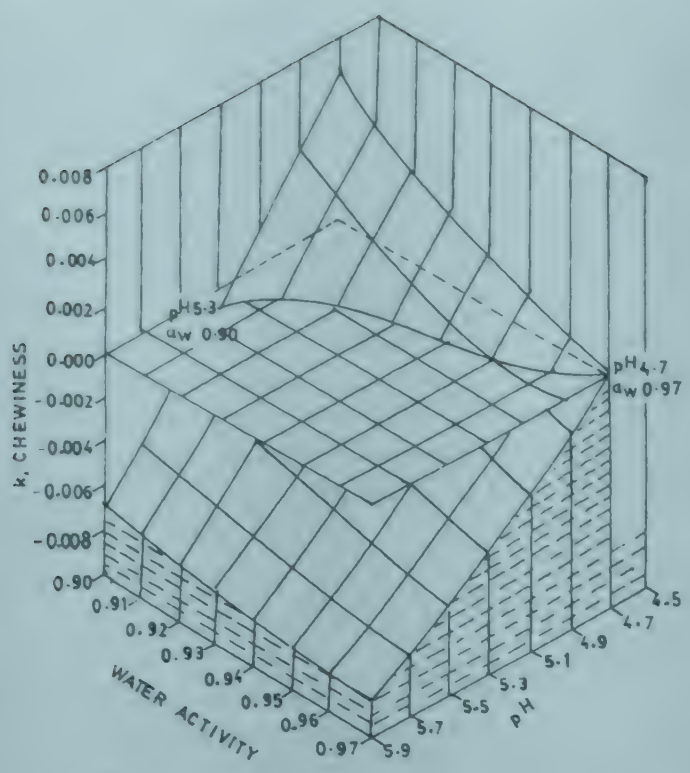

Fig. 7. Response surface, intersected by the plane of zero reaction rate, for change in chewiness reaction rate during storage of fried *paneer* (fixed variable, F-value = 0.5)

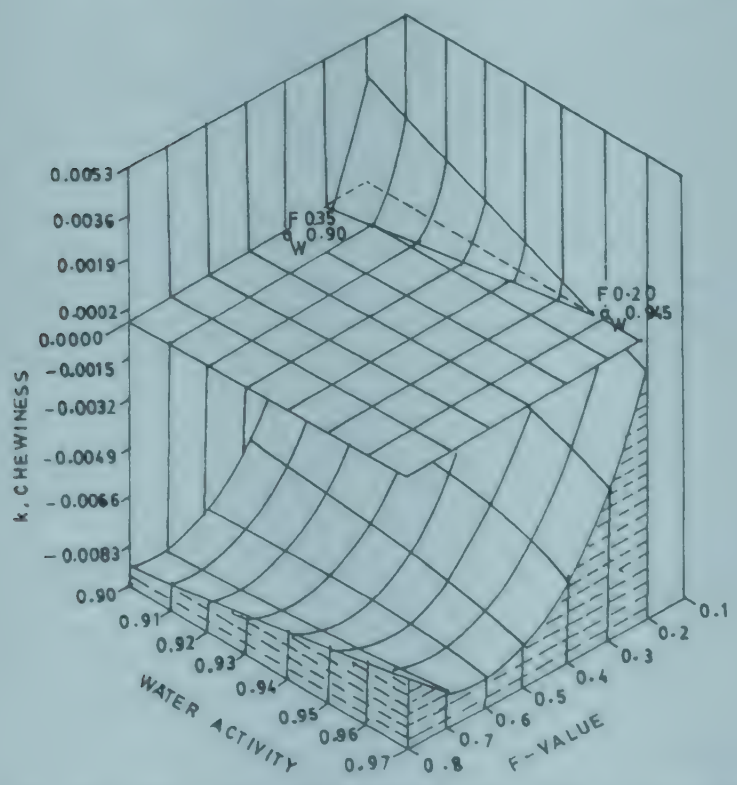

Fig. 8. Response surface, intersected by the plane of zero reaction rate, for change in chewiness reaction rate during storage of fried *paneer* (fixed variable, pH = 5.9)

rearrangement during storage period leading to changes in hardness and chewiness. These changes seem to take place faster with increasing intensity of the parameters a_w, pH and heat treatment. These observations in the present study are of significance in extending the shelf life of canned *paneer* curries. It is recommended to follow the a_w, pH and heat treatments, where the reaction rates of rheological changes tend to be zero.

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RESEARCH PAPER

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Solar Tunnel Drying of Red Chillis (*Capsicum annum* L.)

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The present investigation was aimed to understand the drying efficiency of solar tunnel dryer and to improve the quality of red chillis. An extensive survey of the cultivated regions and drying methods that prevail in Kerala for chilli was conducted. Attappadi area of Palakkad district was identified as the major chilli-growing region in the State. Wet samples of red chillis from two different sampling stations of Attappadi were collected and dried in a tunnel dryer. Both physical and chemical quality analyses of the dried chills were done according to the National and International Standards. The results of quality analyses of both tunnel-dried chilli samples and those chilli samples dried by conventional method, collected from the same sampling locations were compared. Considerable reduction in drying time was noticed in tunnel-dried samples. Improvement in overall quality parameters, cleanliness and texture were noticed in tunnel-dried chilli samples. Optimum conditions required for the drying of red chilli could be identified.

Keywords : Chilli, Drying methods, Solar tunnel-dryer, Quality improvement.

The spice chilli is widely distributed in India. It is an indispensable condiment of every Indian home. This spice stimulates saliva and gastric juice and helps in digestion. It has primary origin in Mexico and a secondary origin in Guatemala. India is the largest producer of chillis in the world contributing 25% of the total world production. Around 97% of the total production of chillis is consumed within the country and only 3% is exported (Murthy 1995). The crop can be grown over a wide range of altitudes from sea level up to nearly 2100 meters (Anon 1995). There are two common species of chillis in India viz. *Capsicum annuum* L. the milder pungent one and *Capsicum fruitascence* L., 'the whole hot peppers'. Ugandan chillis known as 'Mombasa' exported through the port of Mombasa are the most pungent of all and well established in the International trade for their pungency (Suresh 1995). Almost all the varieties of chillis that are cultivated in India as a cash crop belong to *Capsicum annuum* L.

Better retention of colour and higher yield of dry chillis with considerable reduction of breakage of pods and loss of seeds are reported to have been achieved over the traditional method by adopting the improved technology for sun-drying of chillis (Sumathykutty and Mathew 1987). Harvested red chilli contains 72-78% of moisture that should be reduced to 8-10% by drying for the preparation of chillis of commerce. Ripe fruits are harvested at frequent intervals (20-30 days) and dried on floor after heaping in room for one day to maintain the uniform colour. Since the crop is exposed to sunlight for 6-15 days on open floors, the chances of contamination with foreign matter and microbes are inevitable. Mechanical, electrical and solar drying methods for chilli drying are developed recently by various workers (Kachru and Gupta 1993; Anon 1979; Achary 1995).

Ovary wall of chilli fruit is responsible for the production of capsaicin. About 90% of the capsaicin is distributed in the placenta. Oleoresin obtained from chilli is of great commercial importance as a value-added product and as a raw material for medicinal use. 'Capsicum oleoresin' from red chilli has recently entered the composition of ointments for quick relief from pain, swelling and inflammation (Varghese and Natarajan 1998). Colour of chilli is also an important attribute for quality assurance along with pungency and oleoresin. The chief pigment responsible for colour is an alkaloid called capsanthin. Apart from this, α-carotene, β-carotene, xeaxanthin, cryosanthin, lutein and some unidentified xanthophylls are also contributing, varying intensities of colour. In Japan, sun-drying of chillis is followed by mechanical drying, dressing and again sun-drying before packing (Pruthi 1992).

Optimum and standard conditions required for drying of 'sannam' variety of red chilli collected from two different locations of the State of Kerala were evaluated in the present study. A comparative study was also carried out with solar tunnel dried samples and commercially available samples of the same location.

Material and Methods

Sampling locations were identified at Attappadi area in Palakkad district of Kerala State. An extensive survey was conducted in the study area to identify the post-harvest handling practices and technologies for red chilli prevailing in the sampling sites. Freshly harvested red chillis of 'sannam' variety were collected thrice from two different locations of Attappadi during the months of February and March 1998. At each sampling, 50 kg of red chillis was purchased. Samples were collected in clean polyethylene bags and transported to the research centre within 5 h of harvesting. Samples were washed twice in tap water and allowed to drain excess water for half an hour. It was then heaped in a room for 24 h. Length and diameter of wet samples of red chillis were measured. Fresh weight and initial moisture content were also noted.

Solar tunnel dryer : A German made solar dryer (Esper and Muhlbauer 1996) was used to dry red chillis in the present investigation. This solar tunnel dryer developed at the Institute for Agricultural Engineering in the Tropics and Sub-tropics of Hohenheim University, Germany, consists basically of a plastic foil covered flat plate solar air heater, a drying tunnel and

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TABLE 1. OBSERVATIONS ON DRYING CONDITIONS DURING THE DRYING OF CHILLIS IN SOLAR TUNNEL DRYER

	Temperature, °C				Relative humidity, %				Incident solar energy, W/m²/day	
Days dried	Ambient air	S.D.	Dryer	S.D.	Ambient air	S.D.	Dryer	S.D.	P.E. Foil	S.D.
First day	36.0	± 2.17	58.5	± 10.9	51.1	± 6.7	40.2	± 09.7	360.6	± 260.4
Second day	34.3	± 2.62	47.9	± 15.4	54.9	± 4.4	48.3	± 10.4	524.8	± 269.3

small axial flow fans. The entire floor of both solar air heater and the drying tunnel has a length of 17 m (10 m for tunnel and 7 m for heater) and 2 m breadth. Solar air heater and the tunnel are covered with a transparent UV stabilized PE plastic foil, 0.2 mm in thickness with a transmissivity of 92% for visible radiation. The capacity of the tunnel ranges from 60 kg to 200 kg wet fruits depending upon the size of the crop and thickness of the spreading layer. This solar tunnel dryer is installed at the research centre of Sacred Heart College and all the fruit drying experiments were conducted in this dryer.

Drying of chillis : Chilli fruits were spread in one fruit layer on a clean perforated mat kept inside the drying tunnel of the solar tunnel dryer about 2 cm above the surface. No overlapping and clustering of fruits were allowed. Initial temperature and relative humidity of the dryer and ambient air were recorded before drying, using a mercury thermometer and an air guide instrument (USA), respectively. The dryer was closed by steering down the pedal carrying the PE (polyethylene) foiled roof and allowed the red chilli samples to dry. The intensity of solar radiation was measured using a pyranometer from the roof of the dryer. Temperature and relative humidity readings were taken at two-hour intervals between 9 am to 5 pm of the ambient air and the dryer for two days. Incident solar energy was also measured during the drying period. Temperature and relative humidity were measured at the junction of solar air heater and drying tunnel, middle of the tunnel just above the crop and at the outlet of the dryer, respectively. Re-spreading, mixing and heaping were carried out at regular intervals for uniform drying and colour development. Length and diameter of dried chills were measured. Dry weights of the samples were also noted. Calyx of all the produce was removed manually after drying. Random samples were taken in triplicate after drying for the analysis of physico-chemical and quality attributes. These analyses were carried out at the Quality Evaluation and Upgradation Laboratory of Spices Board, Kochi and at the Research Department of Botany, Sacred Heart College, Thevara according to the National and International standards.

Cleanliness parameters (ASTA 1998), Agmark grade specifications for spices (Anon 1996) and chemical parameters such as moisture, oleoresin, capsaicin, colour value, total ash and acid insoluble ash (ASTA 1997) were analysed.

For a comparative evaluation of quality, commercially available dried chilli samples from the same sampling locations were also collected for the analyses of same parameters as adopted for tunnel dried samples.

The data collected were analysed statistically as per the method according to Snedecor and Cochran (1967). The percentage of spice recovery was also calculated.

Results and Discussion

Survey showed that the major chilli cultivation area in Kerala is restricted to Attappadi region of Palakkad district. According to previous reports, Malappuram, Kannur and Kasaragod districts were also contributing a small quantity of chillis (Gangadharan 1999). It was observed that only conventional sun-drying method was commonly practised in Kerala for drying of red chillis.

Results on drying conditions such as drying time, temperature, relative humidity and incident solar radiation recorded during red chilli drying are given in Table 1. In the present investigation, only two days were taken for the optimum drying of red chillis in the solar tunnel dryer. Minimum of 7 days time was required for the chillis to dry by the conventional sun-drying method. According to Bhalerao (1993), sun-drying of red chillis is a time consuming process extended up to 20 days. Open sun-drying has been widely practised in tropical countries, but the method is extremely time-consuming, weather dependent and has the problem of contamination, infestation and microbial attack (Ratti and Mujumdar 1997).

Temperature inside the dryer during drying was always higher than the ambient air due to the green house effect of PE foil. Relative humidity was high in the ambient air when compared to the inside the dryer. There was significant difference in the intensity of incident solar radiation, received on both days of chilli drying. The incident solar energy on the first day was measured as 360.6W/m²/day, resulting in an ambient air temperature of 36°C at a relative humidity of 51.1%. The solar radiation on the second day was 524.8 W/m²/day, but the relative humidity being 54.9%, the ambient air temperature was lowered to 34.3°C. The temperature inside the dryer varied with the ambient conditions, being 58.5°C on the first day and 47.9°C on the second day. Optimum drying temperature of 65°C reduced the drying time by 50% against drying in the sun (Varghese 1995). However, chilli dried beyond a temperature of 55°C resulted in the loss of capsaicin content and slight darkening of the red colour of the produce (Kachru and Gupta 1993).

Table 2 shows the percentage moisture content and spice recovery after drying. The drying time taken for conventional and solar tunnel dryer methods are also given in Table 2. Chillis collected from different sampling locations of Attappadi showed that different drying periods were required for optimum drying by the conventional method, but all the samples dried in two days only in the solar tunnel dryer. Approximately, 31.44% of dried chillis were recovered after the drying of wet red chillis. Percentage of the produce recovered after drying showed no variations among the samples of different localities. In the solar tunnel dried chillis, percentage

TABLE 2. A COMPARATIVE ACCOUNT OF CONVENTIONAL AND SOLAR TUNNEL DRYING OF CHILLIS FROM DIFFERENT SAMPLING LOCATIONS

Sampling stations	Initial moisture content, %		Spice recovery, %		Final moisture content, %		Number of days for optimum drying	
	*C.S.	Dryer	C.S.	Dryer	C.S.	Dryer	C.S.	Dryer
Kottathara	75	75	30.80	31.80	7.60	6.80	7	2
Sholiyoor	75	75	30.50	31.08	8.00	6.80	10	2
Mean	75	75	30.65	31.44	7.80	6.80	8.50	2

Physical characteristics of 'sannam' red chillis

	Length, cm				Diameter, cm			
	Before drying		After drying		Before drying		After drying	
	C.S.	Dryer	C.S.	Dryer	C.S.	Dryer	C.S.	Dryer
Kottathara	7	7	6.2	6	4	4	3.2	3.4
Sholiyoor	7	7	6.2	6	4	4	3.2	3.6

*Commercial sample

TABLE 3. QUALITY ATTRIBUTES OF RED CHILLI SAMPLES COLLECTED FROM DIFFERENT STATIONS DRIED IN SOLAR TUNNEL DRYER AND BY CONVENTIONAL DRYING

Quality parameters	Kottathara 'Sannam'		Sholiyoor 'Sannam'	
Chemical	*C.S	Dryer	C.S.	Dryer
Capsaicin, % by weight	0.20 ± 00.11	0.24 ± 00.22	0.19 ± 00.50	0.21 ± 00.04
Colour value ASTA Clr. Units	56.60 ± 11.90	73.55 ± 22.90	50 ± 11	70.50 ± 10.9
Oleoresin, % by weight	16.44 ± 04.50	16.80 ± 02.24	14 ± 02.30	15.70 ± 03.60
Moisture, % by weight	7.60 ± 01.10	6.80 ± 02.50	8.00 ± 01.91	6.80 ± 01.23
Total ash, % by weight	5.60 ± 01.90	6.07 ± 02.20	6.21 ± 02.20	4.62 ± 01.50
Acid insoluble ash, % by weight	0.08 ± 00.02	0.04 ± 00.01	0.46 ± 00.11	0.01 ± 00.01
Physical				
Whole insects dead, % by count	10 ± 00.2	1.00 ± 00.02	9 ± 04.00	ND
Excreta mammalian, % mg/lb	238 ± 32	ND	108 ± 21.00	ND
Excreta others, % mg/lb	58 ± 21.9	ND	128 ± 22.10	ND
Mold, % by weight	5.60 ± 03.90	ND	6.90 ± 03.90	ND
Insect defiled/infested, % by weight	3.90 ± 00.09	1.70 ± 00.02	3.10 ± 02.00	1.68 ± 00.09
Extraneous foreign matter, % by weight	1.20 ± 00.01	ND	1.87 ± 01.01	ND
Loose seeds, % by weight	0.90 ± 01.01	3.10 ± 0.78	0.30 ± 00.02	5.90 ± 02.32
Broken chillis, % by weight	2 ± 01	1 ± 0.01	ND	1
Damaged and discoloured, % by weight	10 ± 03.30	4 ± 2.40	19 ± 06.98	5 ± 02.65

*Commercial sample

moisture content varied from 6.8 to 9.6. 'Sannam' chillis measured a mean length of 7 cm and a mean diameter of 4 cm before and 6 cm in length and 3.5 cm in diameter after drying.

Results on the quality attributes of the red chilli samples dried in solar tunnel dryer and commercial samples are given in Table 3. The samples dried by the conventional method were contaminated by insects, their excreta, mold and other extraneous foreign matter, irrespective of the sampling location, whereas the samples dried in the solar tunnel dryer were free of such contamination. The capsaicin content was slightly higher in the solar tunnel dried samples. The colour value increased significantly by the tunnel drying. The moisture content was lower in the dryer samples, while oleoresin and ash contents were similar in conventional drying and tunnel drying. The quality parameters such as capsaicin, colour value, oleoresin, moisture and ash contents of both conventional and tunnel dried chills were more or less same. However, the physical qualities of the conventional samples were not within the ASTA limits. The tunnel-dried samples were clean and keeping with the ASTA and Agmark limits of quality.

Conclusion

The results of the present investigation have shown that the method of drying determines the quality of the spice. Chills dried in solar tunnel dryer was hygienic and showed high quality over commercially available chillis. In solar tunnerl dried samples, drying time was highly reduced when compared to the commercial samples. Overall quality of chillis including colour, pungency and texture were also improved by drying in solar tunnel dryer. Moreover, it was possible to ensure the quality of spice produced under the optimum drying conditions of the dryer.

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RESEARCH PAPER

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Studies on Nitrogen Extractability of Defatted Sunflower Meal

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Edible grade meal was prepared from dehulled and defatted sunflower seed kernels and analyzed for proximate principles, polyphenols and phytate and its nitrogen extractability was studied at different variables. The kernels contained 19.8 % proteins, 50.1 % fat, 3.7 % ash, 1.6 % polyphenols and 1.7 % phytate on dry weight basis. Dehulling kernels increased relative concentrations of these components on unit weight basis. A solid : liquid ratio of 1:20 and extraction time of 30 min were found to be the most optimum nitrogen solubilizing parameters. Addition of sodium chloride increased the nitrogen extractability in isoelectric pH range.

Keywords : Sunflower meal, Polyphenols, Phytate, Dehulling, Nitrogen extractability, Defatting, Isoelectric pH.

Sunflower (*Helianthus annuus* L.) appears to have a tremendous potential for meeting the ever increasing demand for oil and proteins by virtue of its high oil content (40-45 %), high oil yield percentage and the relatively high protein content (20-29%) of its meal. Moreover, the oil is considered to be of high quality due to its cholesterol-reducing properties (Bendictus 1980). Sunflower meal is used primarily in ruminant feed, but its nutritional, sensory and functional properties make sunflower meal potentially useful in human foods.

As with many other oilseeds, sunflower contains several undesirable compounds, which affect its utilization as source of dietary proteins. Sunflower seed contains a substantial quantity of hull (>20%), which is sometimes difficult to remove and adds high fibre in the meal, which imparts a dark colour to it (Wan et al. 1979). The phenolic compounds have been a major impediment to the use of sunflower proteins in food products due to their chromophoric properties (Carter et al. 1972). The phytate in sunflower meal (typically more than 3.5%) must also be reduced because of its effect on nutritional and functional properties of proteins (Cheryan 1980).

The nitrogen extractability of sunflower seed is the function of quantity of hulls, polyphenolic compounds and phytate and also the various extraction variables such as solid: liquid ratio, extraction time, pH, ionic strength etc. The process for studying the effects of these parameters is standardized and results are reported in the present paper.

Material and Methods

Dehulling of sunflower seeds : The sunflower seeds (var. 'MSHF-8') were procured from the Department of Agronomy, Marathwada Agricultural University, Parbhani and conditioned to hasten the removal of hulls by using water for 4-5 h. The hulls were separated manually. Then, the kernels were dried to original moisture level at room temperature and percentage of hulls was calculated.

Preparation of defatted sunflower meal (DSM) : The dehulled sunflower seeds were ground in a Waring blender. The oil was extracted by shaking the flour with n-hexane using flour to solvent ratio of 1:3 (w/v) for 16 h at room temperature. The suspension was filtered under vacuum through Whatman No. 3 paper. The residue was dried and ground to 60 mesh size with a laboratory mill (falling number).

Proximate composition : The samples of whole seeds, dehulled seeds and defatted meal were analyzed for crude proteins (N × 6.25), crude fat and ash as per the AOAC (1980) methods.

Determination of polyphenolic compounds : The polyphenolic compounds were determined by the method of Dorrell (1976) and Price et al (1978) with slight modifications. The sample of DSM (200 mg) was suspended in acidified butanol in a ratio of 1:20 (w/v). Acidified butanol was prepared by mixing butanol with 0.005 N HCl (92.8 v/v) and the pH was adjusted to 5.0 with 0.05 N HCl. The extraction was carried out for 15 min at room temperature under constant stirring with a magnetic stirrer. The pH of the slurry was maintained at 5.0 by the addition of 0.5 N HCl. The operation was repeated two to four times. Finally, the supernatants were separated by filtration and combined. To an aliquot (1 ml) of supernatant, 5 ml of vanillin-HCL reagent (1 % vanillin in methanol and 80% concentrated HCl in methanol in the ratio of 1:1) was added and kept at room temperature for 20 min. An absorbance was recorded at a wavelength of 500 nm on spectrophotometer. Simultaneously, a reagent blank was also run. The data were expressed in terms of chlorogenic acid equivalents (mg/100g meal) using a standard curve developed with pure chlorogenic acid (Sigma Chemicals Co. St Louis, Mo).

Determination of phytic acid : The phytic acid was calculated assuming 28.20 % phosphorus in the molecule on the basis of phytic acid phosphorus content. The estimation of phytic acid phosphorus was done using a combination of three methods (Wheeler and Ferrel 1981; Makower 1970; AOAC 1980).

Nitrogen extractability : The nitrogen extractability of DSM at different extraction variables such as solid:liquid ratio (1:10, 1:20, 1:30 and 1:40 w/v), extraction time (20, 40, 60, 80 and 100 min), pH (2, 4, 5, 6, 7, 8, 10 and 12) and ionic strength (0.5 and 1.0 M NaCl) was performed essentially by the procedure described by Dev et al (1986) for linseed meal. The protein precipitability as a function of pH was also determined, simultaneously, using distilled water as a solvent.

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Results and Discussion

The data on proximate composition of sunflower seed and defatted meal (Table 1) show that whole seed contained 19.8 % proteins, 50.1 % fat on dry weight basis. However, it was found to contain a substantial amount of polyphenols (1.6 mg/100 g) and phytate (1.7 mg/100g).

It was observed that removal of seed coat of sunflower seed kernel increased the protein and oil contents by 32.6 and 21.3%, respectively, while the ash content was lowered by 7.6%. Since the hulls contributed about 38.5% portion of the whole seed weight and it contained relatively low levels of proteins and oil, its removal led to an increase in these components on unit weight basis. Dehulling increased the relative concentration of phytate by about 17.6% and of polyphenols by about 6.3%, indicating that these were primarily present in the kernels. It is known that phytic acid reportedly forms a complex with seed coat fractions and as a result, lead to lower concentration of phytic acid in whole seeds. The results are in close conformity to those of Dreher and Holm (1983) and Sayeed and Cheryan (1988). The removal of oil in the defatted meal further increased all these components on unit weight basis. Phytate and polyphenols increased by about 135.8 and 120.0%, respectively, over the whole seed, probably because of removal of major seed components. Protein and ash contents also increased in the proportion of the amount of oil removed.

The data on nitrogen extractability of DSM (Fig. 1) indicate that a solid : liquid ratio of 1:20 and extraction time of 30 min were most optimum, solubilizing about 58.5% nitrogen. The increase in solid ranged from 1:10 to 1:20 had a significant effect on the per cent nitrogen from meal, while further increase beyond 1:20 had not much significant effect on nitrogen extractability. Rivas et al (1981) also reported decrease in nitrogen extractability, when large excess of solvent in the ratio of 1:100 (g/ml) was employed. Similarly, Pawar and More (1993) also reported that a solid:liquid ratio of 1:20 (g/ml) and extraction time of 30 min were most solubilizing parameters of nitrogen solubility of defatted kenaf seed flour in water. As regards the extraction time, it had a very little effect on nitrogen extractibility beyond 30 min of extraction time. The increase in nitrogen extractability was very marginal, indicating that 30 min extraction time was most optimum. Dev et al (1986) reported 30 min optimum time of extraction of linseed nitrogen. The result of the present experiment has suggested that a solid:liquid ratio is an important factor controlling nitrogen extractability.

Nitrogen extractability from defatted sunflower meal within the pH range of 2.0 to 12.0 as illustrated in Fig. 2 shows that a least nitrogen (10.2%) was extracted at pH 5.0. The iso-electric pH of sunflower protein as reported by Sayeed and Cheryan (1988) was 5.0, which fairly coincided to the present observation. At pH 2.0 and at 12.0 about 40.1 and 80.2% nitrogen were extracted, respectively. The results are similar to those described previously for sunflower (Gheyasuddin et al. 1970) and linseed (Dev et al. 1986). Less than 15.0% nitrogen was extracted at pH values from 4.0 to 6.0 but extractability increased on both the sides of isoelectric range of protein being extracted steadily. Rahma and Rao (1979) also reported only 3% nitrogen extractability of sunflower chlorogenic acid-free meal at a pH around 6.0. Madhusudhan and Singh (1983) reported a much broader pH range of least nitrogen solubility (pH 3.0 to 6.0) for demucilaged, defatted and dehulled linseed meal. These differences may be due to varying nature of proteins in defatted oilseeds.

Addition of sodium chloride at two concentrations (0.5 and 1.0 M), and at varying pH was found increased nitrogen extractability in the pH range of 4.0 to 6.0 at each point (Fig. 3). But, there was a slight decrease in nitrogen extractability at pH 2.0 and 7.0 to 12.0. This increase in

TABLE 1. PROXIMATE COMPOSITION OF SUNFLOWER SEED, DEHULLED SEED AND DEFATTED MEAL (% DRY BASIS)[A]

Sample	Crude proteins (N × 6.25),%	Crude fat, %	Ash, %	Poly-phenols, %	Phytic acid, mg%	Hulls, %
Whole seed	19.8	50.1	3.7	1.6	1.7	38.5
Dehulled seed	26.3 (32.6)	60.7 (21.3)	3.4 (7.6)	1.7 (6.3)	2.0 (17.6)	-
Defatted meal	62.1 (206.7)	1.5 (97.0)	7.3 (95.9)	3.5 (120.0)	4.0 135.8)	-

[A] each value represents the average of three independent observations
Figures in parenthesis indicate % increase or decrease

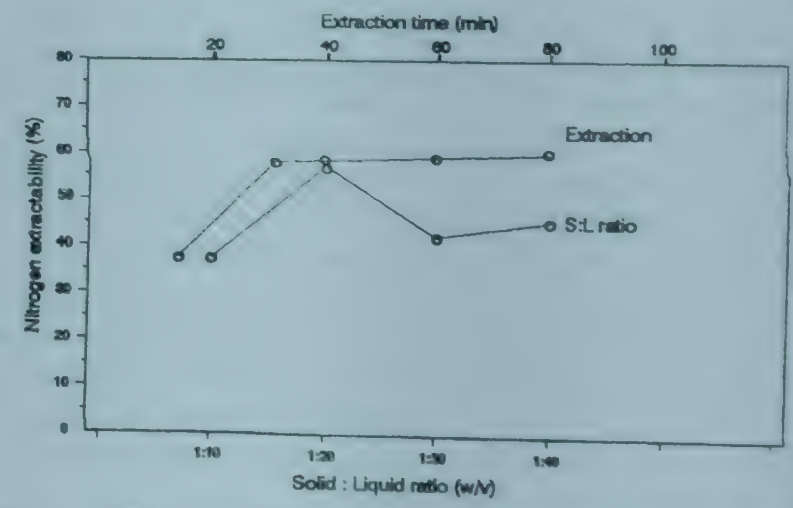

Fig. 1. Effect of solid:liquid ratio and extraction time on nitrogen extractability of defatted sunflower meal

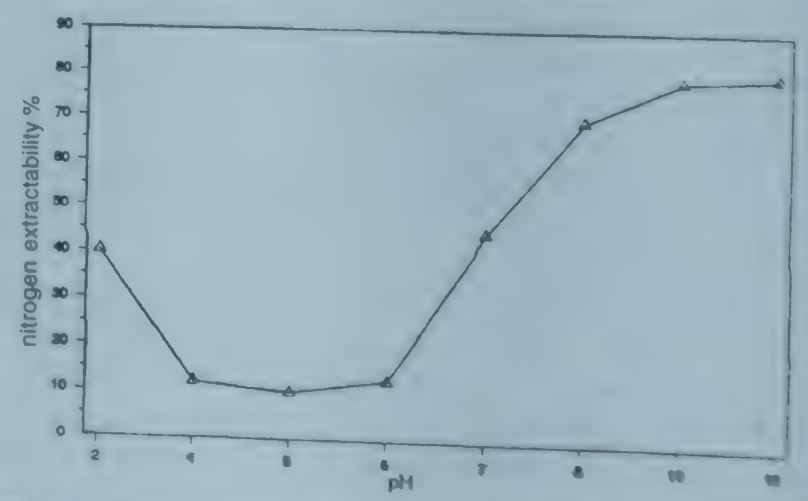

Fig. 2. Effect of pH on nitrogen extractability of defatted sunflower meal

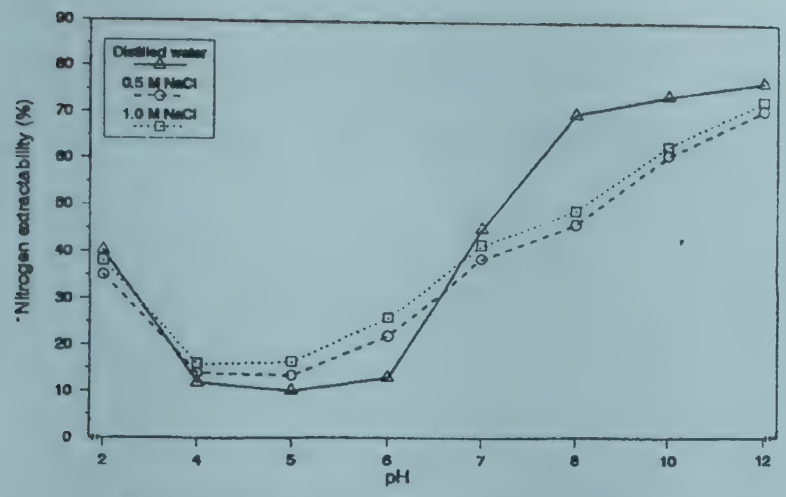

Fig. 3. Effect of ionic strength on nitrogen extractability of defatted sunflower meal at varying pH

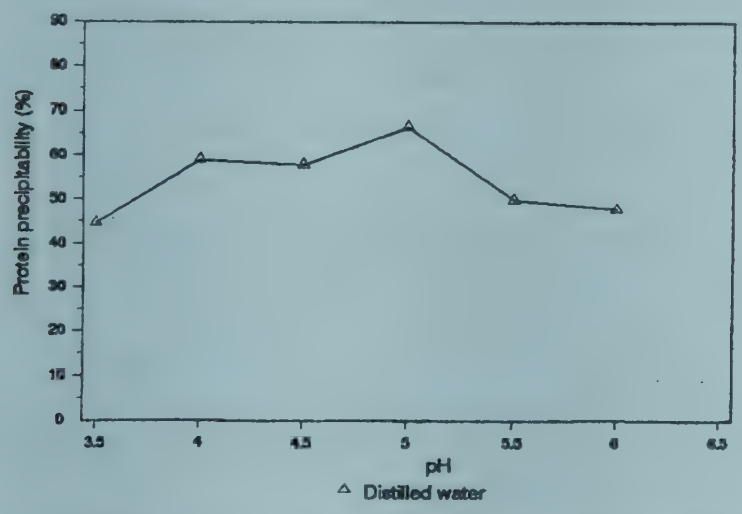

Fig. 4. Effect of pH on protein precipitability of defatted sunflower meal

isoelectric pH range has also been reported by Pawar and More (1993) for kenaf seed flour, in sesame seed (Rivas et al. 1981) and in sunflower (Gheyasuddin et al. 1970; Rahma and Rao 1970). With increase in ionic strength from 0.5 to 1.0 M of NaCl, the nitrogen extractability was found to increase at all pH values. Maximum nitrogen contents in 0.5 and 1.0 M NaCl concentrations extracted were 74.0 and 75.5 %, respectively, at pH 12.0. At extreme pH values i.e. at pH 2.0 and at pH beyond 7.0, the nitrogen extractability was decreased in both the NaCl concentrations. The decrease in acidic pH may be attributed to salting-in effects whereas, in strong alkaline pH, it may be partly due to salting-out effect and partly because of the adverse effect of alkali on protein. In alkaline pH, recemization of some of the amino acids cannot be also ruled out. These parameters might have decreased the nitrogen extractability at these extreme pH values. The results are similar to those described previously for sunflower (Rahma and Rao 1979) and linseed (Madhusudhan and Singh 1983; Dev et al. 1986).

As has been observed earlier that a minimum nitrogen extractability in water was at an isoelectric pH range from 3.5 to 6.5, this region was chosen for studying the protein precipitability. The data shown in Fig. 4 indicate maximum protein precipitability (66.5%) at pH 5.0 and minimum (44.4%) at pH 3.5. Smith et al (1946) observed a maximum precipitation of dispersed linseed protein at pH 5.1. Dev et al (1986) reported maximum protein precipitability of linseed flour at pH 4.1, while More et al (1992) reported the same at pH 4.0 in kenaf seed flour. The results of the present investigation are broadly in agreement with those of Smith et al (1946) but slightly different from those of Dev et al (1986) and More et al (1992). These variations may be due to the varying nature of soluble protein in different oilseeds.

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Synergistic Effect of L-Ascorbic Acid and α-Tocopherol Acetate on the Quality of Ground Chevon During Refrigerated Storage

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Synergistic effect of L-ascorbic acid (AA) and α-tocopherol acetate (TA) on the quality of ground chevon obtained from adult male Beetal × Black Bengal crossbred goat carcasses during refrigerated storage (4±1°C) was studied. The pre-blended ground chevon samples with no additives, 600 ppm AA, 10 ppm TA and 600 ppm AA + 10 ppm TA kept in a refrigerated storage (4±1°C) revealed a synergistic effect of AA + TA to improve different quality characteristics of the meat. AA + TA samples showed a significantly ($P<0.05$) higher WHC %, colour score, odour score and lower pH, cooking loss %, metmyoglobin (MMB) %, TBARS number, PV, FFA % as compared to the control and the samples containing AA or TA alone. All the quality parameters showed a highly significant ($P<0.01$) correlation (r-value) between each other during storage. A strong relationship between TBARS number and MMb% was observed during the storage period and a regression equation ($Y=0.1593 + 0.0173 X$, where Y=TBARS number, X= MMb%) was established. The combined treatment of AA and TA for pre-blending the ground chevon samples extended the shelf life up to 9 days as compared to 3 days in the control sample during the refrigerated storage.

Keywords : Ground chevon, Ascrobic acid, Tocopherol acetate, Pre-blending, Meat quality, Refrigerated storage.

India ranks first in the goat population (about 118 millions), which is about one-fifth of the world population. About 47 million heads of goats are slaughtered annually in the country producing 470 thousand MT of fresh goat meat (chevon), which comprises of about 11.41% of the total meat production (FAO 1994). About 50% of the total fresh chevon produced from adult and spent animals, is very tough and chewy. Therefore, consumers do not prefer to use in their domestic kitchens. On the other hand, such tough meat can be profitably utilised as comminuted meat products. During the process of comminution, the surface area of meat tissue increases, there is more incorporation of oxygen into the meat and the meat is more prone to pigment and lipid oxidation, thereby decreasing nutritional as well as organoleptic qualities (Pearson et al. 1983) and creating a major problem in the development of new convenient meat products and processes (Gray and Pearson, 1987). Lipid oxidation is one of the primary causes of deterioration in the quality of meat and meat products during storage, leading to the development of off-flavour, discolouration, loss of texture, increase drip loss, a decrease in nutritive value and the production of potentially toxic compounds (Buckley et al. 1995; Gray et al. 1996). It is well known that the oxidatively degraded products, especially the free radicals are quite injurious to human health. To prevent such oxidative changes, many chemical antixodants can be used, but for the carcinogenic and other adverse effects of BHA (Ito et al. 1986), BHT (Takahasi 1992), TBHQ (Van Esch 1986) and other synthetic antioxidants. The health conscious meat consumers all over the world now demand fresh meat without any chemical preservative. Many meat research scientists have recently been putting concerted efforts to use natural antioxidants such as vitamin E in minced beef (O'Grady et al. 1998; Formanek et al. 1998), lamb meat (Guidera et al. 1997), pork (Asghar et al. 1991), buffalo meat (Sahoo and Anjaneyulu 1997a,b) and vitamin C in beef steaks (Okayama et al.1987) and ground beef (Mitsumoto et al. 1991). However, literature is silent on the use of vitamin C and E alone or in combination as biological antioxidant in the goat meat system.

Keeping these in view, the present study was envisaged to know the interaction effect of L-ascorbic acid and α-tocopherol acetate in the pre-blended ground chevon during refrigerated storage.

Materials and Methods

Preparation of ground chevon : Goat meat samples (both hind and fore legs) were procured from the freshly slaughtered adult (about 30 months age) male Beetal X Black Bengal crossbred goat carcasses, packed in low density polyethylene (LDPE) bags, kept in insulated ice box and brought to the laboratory within 30 min. The samples were hot boned, trimmed off fat and connective tissues, cut into small chunks and minced in a meat mincer using a 4 mm plate to obtain ground chevon.

Pre-blending with vitamins C and E : Freshly prepared L-ascorbic acid (CDH. LR batch No. 9995, product No. 044006) solution (100 mg/ml) in chilled distilled water and α-tocopherol acetate (CDH-LR batch No. 02097 Product No. 044099) solution (1 mg/ml) in refined soybean oil were used. Ascorbic acid (AA) at 600 ppm and tocopherol acetate (TA) at 10 ppm level, as determined from the earlier experiments (Sahoo and Anjaneyulu 2000) were used for pre-blending of 1 kg GC each in four different experimental batches viz., T_1= GC + noadditive (control), T_2= GC + 600 ppm AA, T_3= GC + 10 ppm TA and T_4= GC + 600 ppm AA + 10 ppm TA. The pre-blended GC of each batch was divided into 200 g aliquot each, packed in LDPE bags, sealed, stored in refrigerated temperature (4±1°C) and examined at two days interval for a storage period of 9 days for different meat quality parameters viz., pH, water holding capacity (WHC), cooking loss (CL), colour score (CS), odour score (OS), metmyoglobin (MMb) content, 2-thiobarbituric acid reacting substances (TBARS) number, peroxide value (PV), free fatty

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acids (FFA) per cent, standard plate count (SPC), psychrotrophic plate count (PPC) and coliforms count (CC).

Analytical methods : pH was recorded by dipping the combined glass electrode of a digital pH meter into the slurry of GC with distilled water (Trout et al. 1992) and water holding capacity (WHC) by centrifugation method (Wardlaw et al. 1973). The cooking loss (CL) per cent of the samples was estimated by recording the weight before and after cooking at 80°C for 20 min (Sahoo 1995). Sensory scores for meat colour and odour were determined by using a 5-point scale (Sahoo 1995). The metmyoglobin (MMb) per cent was estimated as per the method described by Trout (1989). The extraction method described by Witte et al (1970) was followed to determine 2- thiobarbituric acid reacting substances (TBARS) number. Peroxide value (PV) and free fatty acids (FFA) per cent of the meat were determined as per the methods described by Koniecko (1979). To know the microbiological quality of the meat, enumeration of the colony forming units per g of meat was done in respect of standard plate count (SPC), psychrotrophic plate count (PPC) and coliforms count (CC) using Hi-Media plate count agar and violet red bile agar and expressed as $\log_{10}$ $cfug^{-1}$ following the method of APHA (1984).

Statistical analysis : The experiment was repeated thrice. Observed data were subjected to analysis of variance (ANOVA) and critical difference test (Snedecor and Cochran 1994) to compare the mean values of the treatments, storage days and their interaction for vairous quality parameters. Correlation coefficients (r-values) between different quality parameters and a prediction equation (Y=a+bX), where Y=TBARS number, X = metmyoglobin per cent were determined following linear regression analysis, carried out with the help of a statistical software package in a personal computer in the computer section of the university.

Results and Discussion

pH, water holding capacity and cooking loss : The interaction effect of ascorbic acid (AA) and α-tocopherol acetate (TA) on pH, water holding capacity (WHC) and cooking loss (CL) of ground chevon (GC) during refrigerated storage at 4±1°C is presented in Table 1. The treated samples showed significantly lower pH (5.76-5.82) than the control (5.91) sample. There was no significant difference in pH between the samples containing AA and TA. However, the experimental samples treated with AA + TA had significantly lower pH than other treated samples, indicating the interaction effect of the two additives. During storage period, the pH of the meat samples increased linearly ($P<0.05$) from 5.62 on day 1 to 6.06 on day 9. On all the storage days, the combined treatment of AA and TA decreased pH values in the meat samples as compared to the individual treatment of a single additive. The correlation coefficient (r-value) indicated that pH had significant ($P<0.01$) negative correlation with CS and OS and a positive correlation with MMb and TBARS number. It meant that as the oxidative rate of pigment and lipids in the meat samples increased, the pH increased and the CS and OS decreased as evidenced by discolouration and off odour.

The treated meat samples showed significantly higher WHC (10.80-12.60) than the control (8.13) sample. Within the treated samples, AA +TA samples had significantly higher WHC per cent (12.60) as compared to AA or TA samples.

TABLE 1. SYNERGISTIC EFFECT OF ASCORBIC ACID AND α-TOCOPHEROL ACETATE ON pH, WATER HOLDING CAPACITY AND COOKING LOSS OF GROUND CHEVON DURING REFRIGERATED STORAGE AT 4 ± 1°C (MEAN ± SE)

Treatments (n=3)	Storage period, days 1	3	5	7	9	Treatment Mean ± SE (n=15)
			pH			
Control	5.66 ± 0.03	5.79 ± 0.02	5.91 ± 0.03	6.05 ± 0.05	6.17 ± 0.04	5.91 ± 0.02^{c}
AA	5.62 ± 0.01	5.71 ± 0.02	5.77 ± 0.02	5.92 ± 0.03	6.03 ± 0.01	5.81 ± 0.04^{b}
TA	5.63 ± 0.01	5.71 ± 0.03	5.78 ± 0.04	5.90 ± 0.02	6.08 ± 0.04	5.82 ± 0.04^{b}
AA + TA	5.58 ± 0.04	5.66 ± 0.03	5.78 ± 0.01	5.84 ± 0.02	5.96 ± 0.02	5.76 ± 0.03^{a}
Storage Mean ± SE	5.62 ± 0.01^{a}	5.72 ± 0.02^{b}	5.81 ± 0.04^{c}	5.93 ± 0.03^{d}	6.06 ± 0.03^{e}	
			Water holding capacity, %			
Control	13.50 ± 0.87	11.17 ± 0.60	8.83 ± 0.44	4.83 ± 0.17	2.33 ± 0.17	8.13 ± 1.11^{a}
AA	16.33 ± 0.73	13.83 ± 0.93	11.33 ± 0.73	8.67 ± 0.88	4.33 ± 0.33	10.90 ± 1.14^{a}
TA	16.67 ± 1.45	13.33 ± 0.44	10.83 ± 0.93	8.50 ± 0.29	4.67 ± 0.60	10.80 ± 1.14^{a}
AA + TA	17.50 ± 1.44	15.50 ± 0.29	13.17 ± 0.60	10.33 ± 0.44	6.50 ± 0.87	12.60 ± 1.08^{a}
Storage Mean ± SE	16.00 ± 0.67^{a}	13.46 ± 0.53^{b}	11.04 ± 0.55^{c}	8.08 ± 0.65^{d}	4.46 ± 0.50^{e}	
			Cooking loss, %			
Control	46.58 ± 0.84	48.66 ± 0.54	50.16 ± 0.25	51.87 ± 0.30	53.10 ± 0.13	50.07 ± 0.64^{c}
AA	44.27 ± 0.60	44.55 ± 0.70	46.80 ± 0.55	47.76 ± 0.50	48.92 ± 0.54	46.66 ± 0.49^{b}
TA	43.68 ± 0.39	45.09 ± 0.39	46.27 ± 0.21	47.43 ± 0.38	48.62 ± 0.46	46.22 ± 0.48^{b}
AA + TA	41.77 ± 0.35	42.61 ± 0.44	44.04 ± 0.58	45.14 ± 0.62	46.12 ± 0.43	43.94 ± 0.46^{a}
Storage Mean ± SE	44.07 ± 0.57^{a}	45.48 ± 0.69^{b}	46.82 ± 0.68^{c}	48.05 ± 0.76^{d}	49.19 ± 0.78^{e}	

$^{a-e}$ Means with different superscripts in a row or column differ significantly ($P<0.05$)

AA = Ascorbic acid TA = α-tocopherol acetate

There was no significant difference in WHC per cent between AA and TA samples. On all the storage days, AA + TA samples maintained higher WHC per cent. In general, the WHC of the meat samples decreased linearly from 16.00 on day 1 to 4.45 on day 9. The synergistic effect of sodium ascorbate (SA) and TA to increase WHC in ground buffalo meat was found by Sahoo (1995). The WHC had a highly positive correlation with CS and OS and a negative correlation with MMb and TBARS number. This indicated that as the oxidative changes went on increasing in the meat samples during the storage period, the WHC simultaneously decreased. In the present study, the treated samples showed significantly higher WHC and lower pH as compared to control sample. This relationship between pH and WHC in the present study is supported by Schon and Scheper (1960), who found a significant decrease in WHC of veal with increasing pH in the range of 5.4-6.5. Swift and Berman (1959) also observed a negative correlation of WHC with contents of Ca, Mg and K ions.

The cooking loss (CL) per cent of the treated meat samples were significantly lower than the control sample. While comparing within the treated samples, the CL per cent was significantly lower (43.94) in AA +TA samples and there was no significant difference between AA samples (46.66) and TA samples (46.21). During the storage period, the cooking loss was significantly increased on all storage days from 44.07 on day 1 to 49.18 on day 9. The CL showed negative correlation with CS and OS and a positive correlation with MMb and TBARS number. This meant that as the oxidative changes increased in the meat samples, the CL also increased.

Colour stability : The visual colour scores (CS) and metmyoglobin (MMb) contents of the meat samples differed significantly due to the treatments and the storage period (Table 2). The treated samples had significantly higher CS than the control samples. Within the experimental samples, the CS increased in order of TA > AA > AA + TA. During the storage period, the acceptable meat colour (CS=3.00) was maintained up to 9 days by only AA+TA samples, 7 days by both AA and TA samples and only 5 days by the control sample. It was also observed that there was a linear decrease in CS on all storage days. The desired red colour of meat continuously decreased during refrigerated storage, which was observed by previous researchers in buffalo and camel meat (El-kady and Fahmy 1984; Anjaneyulu et al. 1990; Sahoo and Anjaneyulu 1997c). There was a positive correlation of CS with OS (r=0.984) and a negative correlation with MMb and TBARS number, indicating that the pigment and lipid oxidation brought about discolouration changes in the meat samples.

Metmyoglobin (MMb) per cent of all the antioxidant-treated meat samples was significantly lower than the control samples. Within the treated samples, AA +TA showed a significantly ($P<0.05$) lower MMb per cent (40.59) as compared to AA (46.08) and TA (46.14) samples. During the storage period, the acceptable MMb content (53.03) was maintained by only AA+TA samples up to day 9, while that in AA and TA samples up to day 7. There was a continuous linear increase in the MMb content on all storage days from 33.61 on day 1 to 62.24 on day 9. Armstrong (1993) reported that the maximum acceptable limit of MMb content in pork was 60.00%. The MMb content was negatively correlated with CS and OS and positively with TBARS number, showing a relation between the oxidised pigments and the lipid oxidation. The synergistic effects of AA + TA to reduce pigment oxidation in beef (Mitsumoto et al. 1991) and in ground buffalo meat (Sahoo and Anjaneyulu 2000) have also been reported.

Oxidative stability

Odour score : The interaction effect of AA and TA on OS and lipid oxidation of GC samples during refrigerated storage is presented in Table 3. The OS and lipid oxidation parameters viz., TBARS number, PV and FFA differed significantly due to the treatments and the storage days. The OS in all the treated samples was significantly higher than the control samples. Among the treated samples, AA + TA samples

TABLE 2. SYNERGISTIC EFFECT OF ASCORBIC ACID AND a-TOCOPHEROL ACETATE ON VISUAL COLOUR AND PIGMENT OXIDATION OF GROUND CHEVON DURING REFRIGERATED STORAGE AT 4 ± 1°C (MEAN ± SE)

Treatments (n=3)	Storage period, days 1	3	5	7	9	Treatment Mean ± SE (n=15)
			Colour score, 5 pt.			
Control	4.03 ± 0.03	3.50 ± 0.06	3.03 ± 0.03	2.60 ± 0.06	1.73 ± 0.03	2.98 ± 0.21[a]
AA	4.33 ± 0.03	3.97 ± 0.03	3.60 ± 0.06	3.30 ± 0.06	2.73 ± 0.09	3.59 ± 0.15[c]
TA	4.33 ± 0.03	3.83 ± 0.03	3.47 ± 0.09	3.20 ± 0.06	2.47 ± 0.03	3.46 ± 0.17[b]
AA + TA	4.63 ± 0.03	4.13 ± 0.07	3.87 ± 0.03	3.57 ± 0.06	3.07 ± 0.09	3.85 ± 0.14[d]
Storage Mean ± SE	4.33 ± 0.07[a]	3.86 ± 0.07[b]	3.49 ± 0.09[c]	3.17 ± 0.11[d]	2.50 ± 0.15[e]	
			Metmyoglobin, %			
Control	36.10 ± 0.47	46.93 ± 0.96	56.40 ± 0.42	65.87 ± 0.52	70.87 ± 0.83	55.23 ± 3.38[c]
AA	33.60 ± 0.69	37.60 ± 0.38	44.70 ± 0.21	52.97 ± 0.69	61.53 ± 0.43	46.08 ± 2.72[b]
TA	33.70 ± 0.47	36.30 ± 0.32	42.47 ± 0.55	54.73 ± 0.50	63.53 ± 0.43	46.15 ± 3.03[b]
AA + TA	31.07 ± 0.38	33.20 ± 0.31	38.13 ± 1.35	47.53 ± 1.26	53.03 ± 0.81	40.59 ± 2.28[a]
Storage Mean ± SE	33.62 ± 0.58[a]	38.51 ± 1.56[b]	45.43 ± 2.06[c]	55.28 ± 2.04[d]	62.24 ± 1.94[e]	

[a-e] Means with different superscripts in a row or column differ significantly ($P<0.05$)

AA = Ascorbic acid TA = α-tocopherol acetate

TABLE 3. SYNERGISTIC EFFECT OF ASCORBIC ACID AND α-TOCOPHEROL ACETATE ON ODOUR SCORE AND LIPID OXIDATION OF GROUND CHEVON DURING REFRIGERATED STORAGE AT 4 ± 1°C (MEAN ± SE)

Treatments (n=3)	Storage period, days 1	3	5	7	9	Treatment Mean ± SE (n=15)
			Odour score, 5 pt.			
Control	4.23 ± 0.03	3.53 ± 0.03	3.07 ± 0.03	2.70 ± 0.06	1.77 ± 0.03	3.06 ± 0.22[a]
AA	4.50 ± 0.06	4.27 ± 0.03	3.87 ± 0.09	3.47 ± 0.12	2.77 ± 0.18	3.73 ± 0.17[b]
TA	4.47 ± 0.03	4.13 ± 0.03	4.00 ± 0.00	3.53 ± 0.03	2.93 ± 0.12	3.81 ± 0.14[c]
AA + TA	4.67 ± 0.03	4.47 ± 0.03	4.13 ± 0.09	3.80 ± 0.15	3.47 ± 0.09	4.11 ± 0.12[d]
Storage Mean ± SE	4.47 ± 0.05[a]	4.10 ± 0.11[b]	3.77 ± 0.13[c]	3.38 ± 0.13[d]	2.73 ± 0.19[e]	
			TBARS number, mg malonaldehyde/kg			
Control	0.95 ± 0.01	1.06 ± 0.03	1.22 ± 0.04	1.36 ± 0.03	1.58 ± 0.01	1.23 ± 0.06[c]
AA	0.77 ± 0.01	0.84 ± 0.06	0.91 ± 0.05	1.06 ± 0.02	1.21 ± 0.01	0.96 ± 0.04[b]
TA	0.77 ± 0.01	0.87 ± 0.06	0.93 ± 0.01	0.99 ± 0.02	1.14 ± 0.01	0.94 ± 0.04[b]
AA + TA	0.66 ± 0.01	0.72 ± 0.03	0.78 ± 0.01	0.84 ± 0.04	0.99 ± 0.07	0.80 ± 0.03[a]
Storage Mean ± SE	0.79 ± 0.03[a]	0.87 ± 0.09[b]	0.96 ± 0.05[c]	1.06 ± 0.06[d]	1.23 ± 0.07[e]	
			Peroxide value, meq/kg			
Control	1.70 ± 0.06	2.17 ± 0.09	2.63 ± 0.03	3.07 ± 0.07	3.63 ± 0.03	2.64 ± 0.18[d]
AA	1.37 ± 0.03	1.70 ± 0.06	2.00 ± 0.06	2.37 ± 0.09	2.63 ± 0.09	2.01 ± 0.12[c]
TA	1.40 ± 0.06	1.70 ± 0.06	1.90 ± 0.06	2.20 ± 0.06	2.50 ± 0.06	1.94 ± 0.10[b]
AA + TA	1.23 ± 0.03	1.37 ± 0.03	1.53 ± 0.03	1.77 ± 0.03	1.97 ± 0.03	1.57 ± 0.07[a]
Storage Mean ± SE	1.43 ± 0.06[a]	1.73 ± 0.09[b]	2.02 ± 0.12[c]	2.35 ± 0.14[d]	2.68 ± 0.18[e]	
			Free fatty acids, %			
Control	0.37 ± 0.02	0.49 ± 0.02	0.63 ± 0.01	0.79 ± 0.02	1.07 ± 0.03	0.67 ± 0.06[d]
AA	0.28 ± 0.01	0.36 ± 0.03	0.52 ± 0.01	0.66 ± 0.01	0.73 ± 0.03	0.51 ± 0.04[c]
TA	0.28 ± 0.01	0.35 ± 0.01	0.45 ± 0.02	0.62 ± 0.01	0.70 ± 0.02	0.48 ± 0.04[b]
AA + TA	0.24 ± 0.01	0.27 ± 0.01	0.36 ± 0.02	0.52 ± 0.01	0.59 ± 0.02	0.40 ± 0.03[a]
Storage Mean ± SE	0.29 ± 0.02[a]	0.37 ± 0.02[b]	0.49 ± 0.03[c]	0.65 ± 0.03[d]	0.77 ± 0.06[e]	

[a-e] Means with different superscripts in a row or column differ significantly (P<0.05)
AA = Ascorbic acid TA = α-tocopherol acetate

had the most pleasant odour and the OS decreased in order of AA + TA > TA > AA > control. The pleasant odour (OS = 3.0) of meat samples was maintained by AA +TA up to day 9, by both TA and AA up to day 7 and by control sample up to day 5. During the storage period, the OS decreased linearly on all storage intervals from 4.46 on day 1 to 2.73 on day 9. Sison et al (1980) also found a continuous increase in off odour of meat, as the refrigerated storage time progressed. Similar observations were also made in ground buffalo meat (Sahoo and Anjaneyulu 1997c). There was a significant (P<0.01) positive correlation of OS with WHC, CS and a negative correlation with pH, CL, MMb, TBARS number, PV, FFA, SPC, PPC and CC. This indicated the bacterial degradation and oxidative changes in the meat was responsible for off odour development in the meat.

TBARS number : TBARS numbers of all the treated meat samples were significantly lower than the control sample. The synergistic effect of AA and TA for lowering the TBARS number was evidenced by a significantly lower value in the AA + TA sample as compared to AA or TA sample. There was no significant difference between AA and TA samples. The acceptable limit of TBARS number (1.00) in the present study was maintained by AA + TA samples till day 9 as compared to TA and AA up to day 7. Earlier research works indicated that the minimum threshold value or acceptable limit of TBARS number of cooked meat products during storage was 0.50 to 1.0 mg malonaldehyde/kg as detected by a trained panel (Tarladgis et al. 1960). The inexperienced panel detected in a TBA range of 0.6-2.0 (Greene and Cumuze 1982) in beef samples. Watts (1962) reported the threshold value of TBA at 1-2 mg/kg for rancidity in meat. During storage period, TBARS number increased consistently on all storage days from 0.788 on day 1 to 1.230 on day 9. Previous researchers also found that vitamins C and E acted synergistically to minimise lipid oxidation in beef loin steaks (Okayama et al. 1987), ground beef (Misumoto et al. 1991), ground buffalo meat (Sahoo 1995) and in turkey meat (Brunn-Jensen 1997).

There was a significant (P<0.01) positive correlation of TBARS number with pH, CL, MMb, PV, FFA, SPC, PPC, CC and a negative correlation with WHC, OS,CS. A strong relationship between MMb accumulation and lipid oxidation in GC during refrigerated storage was observed (Fig. 1). A prediction equation ($Y=0.1593+0.0173\ X$, $R^2=0.8557$) was established, where Y=TBARS number and X=MMb per cent. Similar strong relationship between pigment and lipid oxidation was also reported in beef muscles (Faustman et al. 1992; ground buffalo meat (Sahoo and Anjaneyulu 1997c) and in sliced ham (Andersen et al. 1990). In this context, Harel and

Kanner (1985) reported that there was a reaction of MMb with endogenously produced H_2O_2 to form H_2O_2 - activated MMb which could catalyse lipid oxidation.

Peroxide value and free acids : A similar trend was observed in PV and FFA of the meat. All the treated samples had significantly lower PV as compared to control sample and again, a significant difference of PV was seen among the three treated samples between each other. A significantly ($P<0.05$) lower PV was noticed in the AA + TA samples. PV of the meat samples decreased in order of AA > TA > AA + TA samples. During the refrigerated storage period, PV was found to increase consistently on all the storage days. PV had a significant ($P<0.01$) positive correlation with MMb percent and TBARS number, indicating that the increase of oxidative changes in meat samples also increased the PV. It had a significant negative correlation with CS and OS. In a similar manner, the treated GC samples also showed a significantly low FFA content as compared to the control sample. The FFA content in meat sample decreased in order of AA > TA > AA + TA samples. Kanatt et al (1998) observed that α-tocopherol treatment to chicken meat could significantly lower the FFA content during chilled storage. Data on storage study revealed that FFA increased consistently on all storage days. Previous workers also noticed a rise in the FFA content of refrigerated stored chicken meat (Sharma et al. 1982; Babu et al. 1994). This may be due to bacterial degradition of meat lipids by the lipolytics. FFA had a positive correlation (r-value) at $P<0.01$ with MMb and TBARS numbers and a negative correlation with CS and OS. This indicated that increase in FFA content in meat also increased the oxidative changes and decreased the pleasant odour and desired colour of the meat.

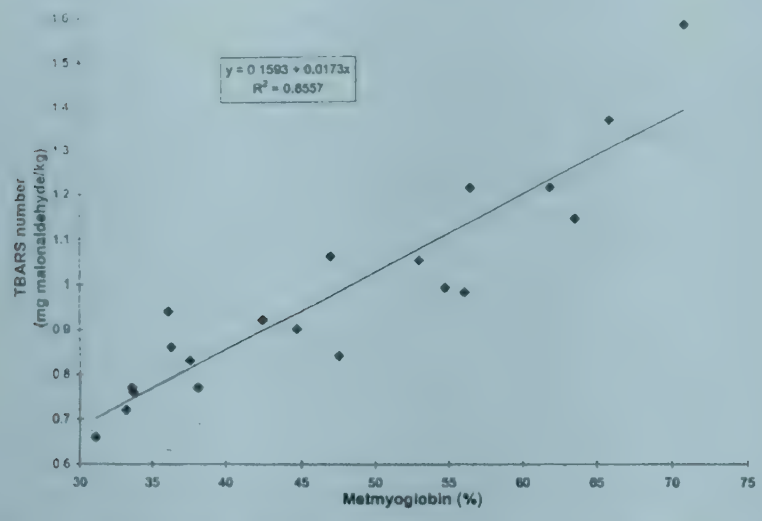

Fig. 1. Relationship between metmyoglobin accumulation and lipid oxidation in ground chevon pre-blended with ascorbic acid and α-tocopherol acetate during refrigerated storage at 4±1°C

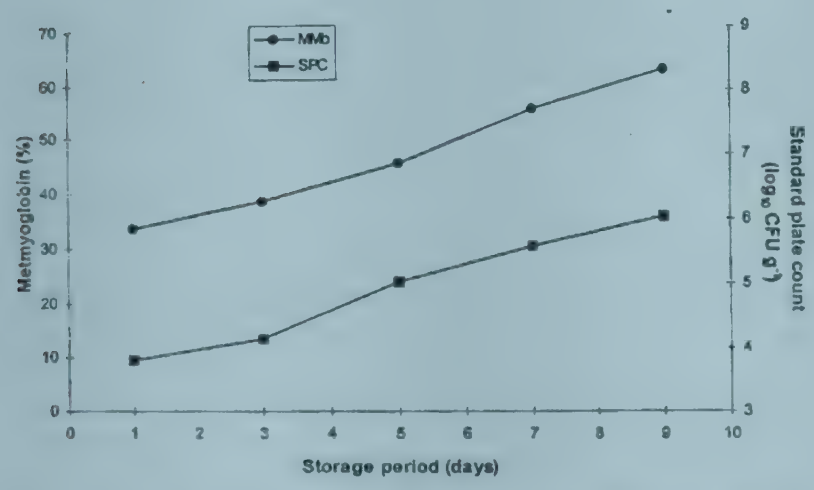

Fig.2. Changes in metmyoglobin content and microbial load in ground chevon pre-blended with ascorbic acid and α-tocopherol acetate during refrigerated storage at 4±1°C

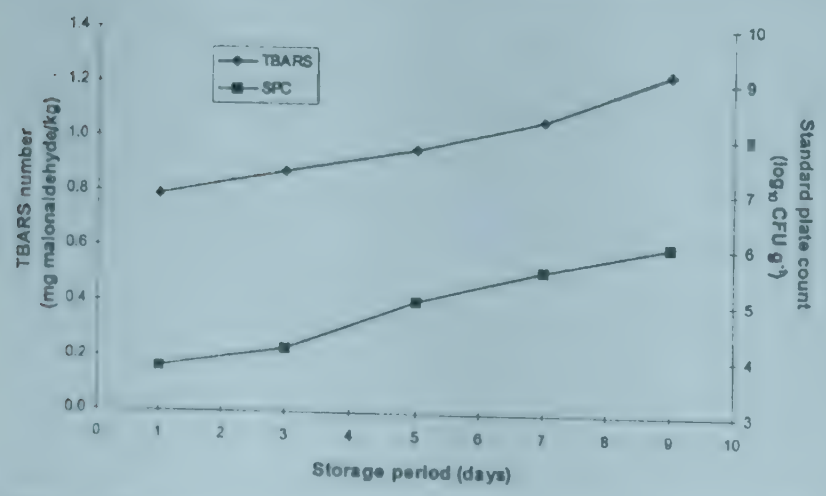

Fig.3. Changes in TBARS number and microbial load in ground chevon pre-blended with ascorbic acid and α-tocopherol acetate during refrigerated storage at 4±1°C

Kanatt et al (1998) observed that α-tocopherol treatment to chicken meat could significantly lower the FFA content during chilled storage. Data on storage study revealed that FFA increased consistently on all storage days. Previous workers also noticed a rise in the FFA content of refrigerated stored chicken meat (Sharma et al. 1982; Babu et al. 1994). This may be due to bacterial degradition of meat lipids by the lipolytics. FFA had a positive correlation (r-value) at $P<0.01$ with MMb and TBARS numbers and a negative correlation with CS and OS. This indicated that increase in FFA content in meat also increased the oxidative changes and decreased the pleasant odour and desired colour of the meat.

Microbiological quality : There was no significant difference between control and treated samples or within the treated samples in respect of SPC, PPC and CC. Present study revealed that the microbial count consistently increased on all storage days in all the bacterial groups. The SPC and PPC increased from log 3.82 and log 3.59 on day 1 to log 6.04 and log 5.96 on day 9 of the storage period, respectively. On the other hand, the coliforms count increased from log 2.05 on day 1 to 3.18 on day 9. All the above microbiological quality parameters showed a significant positive correlation (r-value) with MMb and TBARS numbers and a negative correlation with CS and OS. This indicated, that increase of the microbial load in meat samples also caused increase in oxidation changes and a decrease in the desired colour and odour of the meat. There was a relationship between SPC and MMb content (Fig. 2). As the SPC increased on the storage days, the MMb per cent also increased. Similar observations were also made in the relationship of SPC and TBARS number (Fig. 3). Previous research workers also reported the role of bacteria causing discolouration in meat (Schweigert 1956; Butler et al. 1953; Costilow et al. 1955). It is believed that the oxidative changes of meat is related with the bacterial activity. Neil and Hastings (1925) reported that *Pneumococci* bring about oxidation by production of peroxide or similar compounds. Smith and Alford (1969) reported that organisms like *Pseudomonas ovalis, Micrococcus freuden-seichii* and strains of *Streptomyces* produced peroxide, carbonyl, aldehyde and ketones, causing off odour and off flavours in lard.

Conclusion

Based on the above results of colour, odour, MMb and TBARS number, it may be concluded that there is a strong synergistic effect of AA and TA to minimise the oxidative

changes of meat and there is an extension of shelf life up to day 9 by AA + TA sample as compared to day 3 in control sample during the refrigerated storage.

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RESEARCH PAPER

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Packaging and Storage Studies on Commercial Varieties of Indian Chillis (*Capsicum annum* L.)

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Studies carried out to design a suitable consumer package for 'Guntur' and 'Byadigi' varieties of chillis revealed that moisture contents of 14.00 and 11.2 %, respectively were critical for facilitating mould growth in them. But initial moisture contents of 10.45 % at 64% RH and 9.60% at 57% RH were found critical for storage of these varieties, repsectively. Under tropical conditions, 300 gauge High Density Polyethylene (HDPE) films were suitable for packaging 'Guntur' and 'Byadigi' whole chillis in unit packs of 250 g. However, the Metallised Polyester Polyethylene (MPP) stored samples retained higher colour than HDPE in both the conditions regardless of varieties. The MPP and HDPE packagings were not suitable for storage of 'Guntur' and 'Byadigi' chillis in accelerated conditions (90%, RH-38°C). The discoloration of the red pigments of chillis during storage was greatly influenced by moisture and temperature. The capsaicin content showed a decreasing trend irrespective of storage conditions, packagings and varieties. Under identical packaging and storage conditions, 'Guntur' chillis had better shelf life than 'Byadigi' chilli.

Keywords : 'Guntur' chillis, 'Byadigi' chillis, Capsaicin, Pungency, Packaging and storage.

Chillis, were introduced by the Portuguese in Goa in the middle of 17th century and since then, they had spread rapidly throughout the country. India is contributing nearly 25% of the total world production of chillis. (Sivadasan and Devananda Shenoy 1995).

Chillis are cultivated as one of the important cash crops throughtout India. On an average, they occupy an area of 8.315 lakh hectares with a production of 8.218 lakh tonnes of dry chillis per annum. During 1999-2000 India earned 216.61 crores from an export of 61,253 tonnes, (Spice Board 2000).

The principal colouring pigment of dried chillis is carotenoid capsanthin, a precursor of vitamin A. The active principle of pungency is capsaicin. The chillis are valued for their colour and pungency. High coloured and low capsaicin containing chillis like 'Byadigi' are preferred in the preparation of oleoresins. High pungency chillis are preferred in the manufacture of pure capsaicin or in the preparation of pharmaceuticals like pain balm. Hence, these two quality parameters determine the price of dried chillis. Factors that affect these two parameters of quality are, climatic conditions, period of storage, harvesting time, apart from varietal differences.

Literature survey has shown that colour retention is affected by the stage of ripeness at harvest (Lease and Lease 1956 a). Also, colour retention is affected by the addition of the antioxidant BHA to dry ground chillis (Lease and Lease 1956 b). The processing of 'Bola' and 'Agridulce' varieties showed variety dependent differences in pigments stability. The drying and milling do not affect all the pigments equally. The yellow pigments are highly stable during processing (Minguez-Mosquera 1993).

Mahadevaiah et al (1976) studied the packaging and storage of ground and whole 'Guntur' chillis using LDPE and HDPE packagings. The authors monitored only the colour change as quality parameter. But, the pungency is also a quality parameter of chillis. Khurana et al (1997) studied the capsaicin and colouring matter of different cultivars of chillis dehydrated by different methods.

In India, the dried chillis are stored in gunny bags for marketing. During this period, the deterioration in quality is more due to improper packaging and storage. The information available on quality parameters like colour and capsaicin in 'Guntur' and 'Byadigi' chillis during storage is very limited.

The present investigation was undertaken to study the possible effect on quality with respect to colour and capsaicin in 'Guntur' and 'Byadigi' dried whole chillis packed in HDPE and MPP flexible pouches and stored at different environmental conditions.

Materials and Methods

Chillis : Commercial 'Guntur' and 'Byadigi' chillis were procured from the local market. Broken chillis and foreign matter were separated. The cleaned chillis were used in the present studies.

Packaging materials : High Density Polyethylene (HDPE-75 μm), Metallised Polyester Polyethylene (MPP). Metallised Polyester Polyethylene (MPP-12 μm) and Low Density Polyethylene (LDPE-37 μm) were used in the present studies.

Analysis : The water vapour transmission rates of the HDPE and MPP packagings used were determined (IS:1060 part II-1969) and are expressed as g/m²/day at 90% RH and 38°C. Moisture was determined by toluene distillation method using 10 g ground chillis (ASTA 1985). Colour was extracted from 3 g ground chillis by subjecting to Soxhlet extraction for 16 h using acetone. The extract was made up to 50 ml with acetone. One millitre of this solution was further diluted to 50 ml with acetone and absorbance was measured at 458 nm using a Spectronic 20 (Baush & Lomb) colorimeter. The colour

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of the dilution was estimated using the absorbance and multiplied by 6.1 to obtain the colour value on raw material basis (ISI 1979 a). Capsaicin was determined according to ISI (1979 b) method. Depending on the capsaicin content, 10-50 µl stock solution was applied as a thin streak covering the entire width of a 2 × 20 cm strip of Whatman No. 3 paper and the spots developed by ascending chromatography in methanol-buffer (60:40 v/v) solvent for 1 h. The strip was dried in air and passed through Gibb's reagent (0.1 % solution of 2,6 dichloro p-benzoquinone-4-chloromine in acetone), air-dried again and sprayed with buffer (a solution containing 3.1 g boric acid and 3.7 g potassium chloride in 100 ml of distilled water adjusted to pH 9.6 with 1N sodium hydroxide). The paper strip was kept in a dark chamber for 30 min. for colour development. The blue spot formed was cut off and eluted with methanol and the eluent was made up to 10 ml and its absorbance was recorded at 615 nm in a Spectronic-20 colorimeter against a reagent blank eluted from the corresponding area of a filter paper strip run simultaneously without sample. The per cent of capsaicin content was calculated by using $E_{1cm}^{1\%} = 640$ at 615 nm.

Sorption isotherm studies : The moisture sorption behaviour of the chillis was studied at 27°C by exposing 'Guntur' and 'Byadigi' chillis (10 g) in petri dishes to different relative humidities (RH), ranging from 11 to 92%, that were built in desiccators using appropriate salt solutions (Rockland 1960). The equilibrated chilli samples were periodically (once in 8 days) weighed, till they attained constant weight or showed signs of mould growth, whichever was earlier. During equilibration, the chilli samples were visually examined for surface colour, texture and mould growth. The equilibrium moisture content (EMC) at different RHs was calculated by adding or subtracting percentage gain or loss to initial moisture content of the chilli samples, as determined by the toluene distillation method (ASTA 1985).

Packaging and storage studies : 'Guntur and 'Byadigi' chillis (250 g) were put in unit pouches (12 × 20 cm) made from HDPE and MPP and heat-sealed. The unit packs were exposed to accelerated (90±2% RH, 38°C), ambient (65% RH, 27°C) and controlled (refrigerator, 4-5°C) conditions. The unit packs were withdrawn at an interval of 45 days over a period of 180 days and visually evaluated for surface colour, texture and mould attack and chemically analysed for moisture, colour and capsaicin content.

Statistical analysis of data : The moisture, colour-value and capsaicin estimated in triplicate for 'Byadigi' and 'Guntur' chillis were subjected to analysis of variance pertaining to $2 \times 2 \times 4 \times 2 \times 3$ factorial design (2 temperatures, 2 packagings, 4 storage periods and 3 replicates) (Montgomery 1976), followed by Duncan's New Multiple Range Test (Harter 1960) to segregate means for the effect of packaging, temperature and storage period.

Results and Discussion

Water Vapour Transmission Rates (WVTR) were high in HDPE (1.9) than MPP (0.8) packagings materials.

Sorption studies on 'Guntur' chillis : Moisture sorption studies at 27°C and at different RHs indicated that the whole chillis with an initial moisture content of 9.0% equilibrated to an RH of 58% (Table 1). The sorption isotherm of product is typical sigmoid type and exhibited steep rise above 70% RH (Fig. 1). Generally, it is recognized that the product, which equilibrates to RH of 70 % and above is prone to rapid physico-chemical changes in the product (Mahadevaiah et al. 1976). Hence, the moisture content corresponding to 70 % RH will be 10.5% a critical moisture content of the product. At 75% RH with an equilibrium moisture content of 14.0%, the product turned intense black in colour, soft texture and developed mould growth.

Sorption studies on 'Byadigi' chillis : The sorption behavior of 'Byadigi' chillis (Fig. 2) was almost similar to that the 'Guntur' chillis. The 'Byadigi' chillis with an initial moisture content of 11.2% equilibrated to an RH of 62%, was the critical moisture (Table 1) content of the product. At 75% RH with an EMC of 15.3%, the product turned intense black in colour and developed mould growth.

TABLE 1. MOISTURE HUMIDITY RELATIONSHIP OF CHILLI VARIETIES AT 27°C

Relative humidity, %	% Equilibrium moisture content (EMC) on dry weight basis 'Guntur'	'Byadigi'
11	2.50	3.00
22	4.60	5.00
32	5.60	6.00
44	6.60	7.10
57	9.00	9.60
64	10.50	11.20+
75	14.00+	15.30++
86	24.00++	25.30+++
92	26.20++++	27.00++++

+ slightly soft
++ soft and mould growth
+++ very soft and mould growth
++++ soggy and heavy mould growth

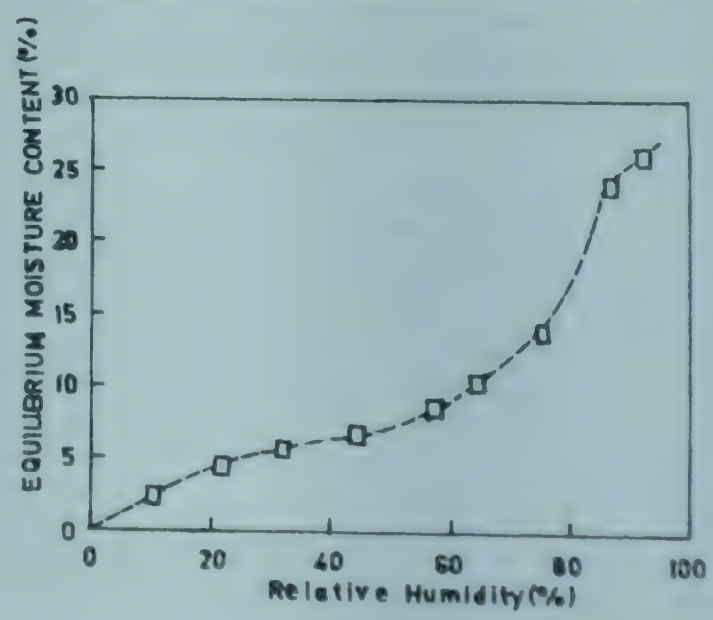

Fig. 1. Humidity moisture relationship of 'Guntur' dried (whole) chillis

Storage studies on 'Guntur' chillis : The effect of packaging, temperature conditions and storage periods for 'Guntur' chillis is given in Table 2. With respect to moisture, HDPE showed significantly higher value than MPP, ambient condition gave significantly higher value than refrigerator and 45 days storage showed significantly lower value than 90, 135 and 180 days storage values, which were comparable. With respect to colour values, the effect of packaging and storage conditions showed the same pattern as 'Byadigi' chillis but the storage periods sshowed ignificantly lower values with the advancement of storage days. With respect to capsaicin, both the packaging and storage conditions showed the same pattern as those of 'Byadigi' chillis. The storage periods showed significantly lower values with the advancement of storage days.

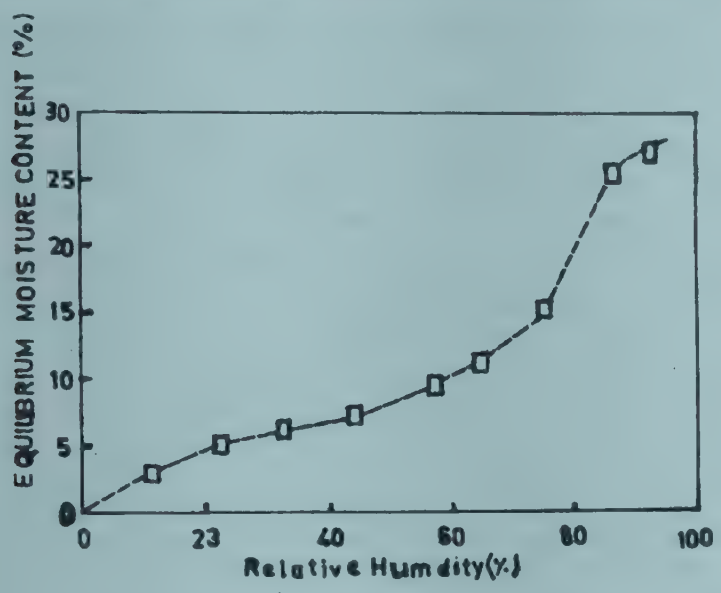

Fig. 2. Humidity moisture relationship of 'Byadigi' dried (whole) chillis

The chillis stored at 90% RH and 38°C had turned intense black in colour and showed almost negligible colour value in both the packagings at the end of 45 days. Chillis analysed after 135 days had almost no colour value but the capsaicin content was comparable with the control and those stored at ambient conditions. The chillis had visible mould growth, musty smell and hence, the samples were discarded. The colour of chillis turning intense black may be due to the non-enzymatic type of browning reaction under high temperature and humidity conditions (Krishnamurthy and Natarajan 1973).

Storage studies on 'Byadigi' chillis : The effect of packaging, temperature and storage periods for 'Byadigi' chillis is given in Table 3. With respect to moisture, HDPE showed significantly higher value than MPP; temperatures gave comparable values and 180 days storage value was significantly lower than 45, 90 and 135 days values, which were comparable. With respect to colour value were MPP showed significantly higher value than HDPE, refrigerator conditions showed significantly higher value than ambient condition and 45 days

TABLE 2. 'GUNTUR' CHILLIS - STATISTICAL SIGNIFICANCE OF MEAN VALUES FOR EFFECT OF PACKAGING, TEMPERATURE AND STORAGE PERIOD

Storage period, days	Packaging HDPE	Packaging MPP	Temperature Refrigerator	Temperature Ambient	Overall mean Storage period (Significance)
Moisture, %					
45	9.20	9.20	9.20	9.20	9.20^{a}
90	9.55	9.20	9.20	9.55	9.38^{b}
135	9.45	9.25	9.15	9.55	9.35^{b}
180	9.45	9.10	9.00	9.55	9.28^{b}
Overall mean	9.41^{m}	19.19^{l}	9.14^{x}	9.46^{y}	$SE_m \pm 0.0577$
(Significance)					(32 df)
Colour value					
45	2267	2716	2631	2352	1176^{d}
90	2034	2276	2372	1938	969^{c}
135	1941	2168	2255	1854	927^{b}
180	1895	2134	2228	1802	90^{a}
Overall mean	2034^{l}	2324^{m}	2372^{y}	1986^{x}	$SE_m \pm 0.9000$
(Significance)					(32 df)
Capsaicin, %					
45	0.38	0.38	0.35	0.40	0.38^{d}
90	0.32	0.34	0.35	0.30	0.33^{c}
135	0.32	0.32	0.35	0.28	0.32^{b}
180	0.28	0.28	0.32	0.24	0.28^{a}
Overall mean	0.32^{l}	0.33^{l}	0.34^{y}	0.31^{x}	$SE_m \pm 0.0006$
(Significance)					(32 df)

SE_m (d_f) –Standard error of the means (degree of freedom) Any two means carrying different superscirpts, l, m or x, y in rows and a,b,c,d in columns differ significantly ($P \leq 0.05$)

TABLE 3. 'BYADIGI' CHILLIS - STATISTICAL SIGNIFICANCE OF MEAN VALUES FOR EFFECT OF PACKAGING, TEMPERATURE AND STORAGE PERIOD

Storage period, days	Packaging HDPE	Packaging MPP	Temperature Refrigerator	Temperature Ambient	Overall mean Storage period (Significance)
Moisture, %					
45	9.90	9.90	9.90	9.90	9.90^{b}
90	10.95	9.95	9.80	10.20	10.00^{b}
135	9.60	9.50	9.65	9.50	9.55^{b}
180	9.55	8.70	9.30	9.05	9.12^{a}
Overall mean	9.78^{m}	9.51^{l}	9.66^{x}	9.63^{y}	$SE_m \pm 0.2387$
(Significance)					(32 df)
Colour value					
45	4879	5580	5321	5138	5230^{b}
90	3368	3798	3989	3177	3583^{a}
135	3228	3744	3915	3057	3486^{a}
180	3199	3680	3900	2978	3439^{a}
Overall mean	3670^{l}	4200^{m}	4280^{y}	3590^{x}	$SE_m \pm 120.20$
(Significance)					(32 df)
Capsaicin, %					
45	0.26	0.24	0.26	0.24	0.25^{d}
90	0.20	0.20	0.20	0.20	0.20^{c}
135	0.18	0.18	0.19	0.16	0.18^{b}
180	0.14	0.15	0.15	0.14	0.15^{a}
Overall mean	0.20^{l}	0.19^{l}	0.20^{y}	0.19^{x}	$SE_m \pm 0.0006$
(Significance)					(32 df)

SE_m (d_f) –Standard error of the means (degree of freedom) Any two means corresponding different superscirpts, l, m or x, y in rows and a,b,c,d in columns differ significantly ($P \leq 0.05$)

storage period showed significantly higher value than 90, 135 and 180 days storage values, which were comparable. With respect to capsaicin, both the packaging were comparable, refrigerator conditions showed significantly higher value than ambient condition and all the storage periods showed significantly lower values.

The chillis stored at 90% ± 2 RH and 38°C had turned intense black in colour and showed almost negligible colour value in both the packagings at the end of 45 days. Chillis analysed after 135 days storage period had almost no colour value but the capsaicin content was comparable with the control sample and those at ambient conditions. The chillis had visible mould growth and musty smell. Hence, the samples were discarded.

Conclusion

Sorption isotherm studies indicated that moisture contents of 10.5% (ERH 64%) for 'Guntur' and 9.6% (ERH 57%) for 'Byadigi' chillis were quite safe for the storage at ambient conditions. At moisture level of more than 14.0% (RH 75%) for 'Guntur' and 11.2% (RH 64%) for 'Byadigi' chillis induced mould growth. From the sorption studies, it was concluded that the 'Guntur' chillis had better sorption behaviour than 'Byadigi'. This might be due to the textural differences among the varieties.

The 'Guntur' and 'Byadigi' chillis (250 g each) packed in HDPE and MPP pouches had better colour even after 180 days at 4-5°C (refrigerator) than at ambient conditions. However, the retention of colour in MPP pouches was better than HDPE pouches at 4-5°C (refrigerator) and at ambient conditions irrespective of variety differences. Under accelerated (90% RH 38°C) conditions, shelf life for 'Guntur' and 'Byadigi' chillis in both the packagings was less than 45 days (Data not included in Tables 2 and 3). The capsaicin content gradually decreased in 'Guntur' and 'Byadigi' chillis (in both the packagings) under control, ambient and accelerated conditions. At identical packaging and storage conditions, 'Guntur' chillis had better shelf life than 'Byadigi' chillis.

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RESEARCH PAPER

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Studies on Development and Storage Stability of Instant Vegetable *Pulav* Mix

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Instant vegetable *pulav* mix prepared from freeze-thaw dehydrated rice, fried peas, fried potatoes, spices and vanaspati and packed in polypropylene (PP) pouches remained stable for 12, 9 and 6 months, while in paper-aluminium foil-polyethylene (PFP) laminate packs it remained stable for 12, 12 and 9 months at 0°C, room temperature (15-34°C, RT) and 37°C respectively. During storage, peroxide value, thiobarbituric acid value and free fatty acids increased, while total carotenoids and chlorophyll contents decreased. Off-flavours resulting from lipid degradation and losses in carotenoids and chlorophyll contents were the major causes of decreased sensory acceptance. Instant vegetable *pulav* mix was most stable at 0.33 a_w.

Keywords : Instant vegetable *pulav*, freeze-thaw dehydration, Storage changes, Proximate composition, Packaging materials, reconstitution.

In India, many dehydrated convenience foods based on cereals, pulses, vegetables and spices have been developed mainly as operational pack rations for our Armed Forces (Arya and Thakur 1986, Vidyasagar et al. 1991; Jayaraman et al. 1991; Patki and Arya 1997). These rations are mainly ready-to-eat or instant convenience food products packed in a manner that troops unaccustomed to cooking are able to prepare palatable and nutritious meals, just by warming or by mixing with hot water. Previously, instant vegetable *pulav* mix had been developed, requiring 10-15 min boiling for reconstitution (Mathur et al. 1972; Bhatia et al. 1964). Despite providing considerable convenience, this product did not receive acceptance from Armed Forces because it took longer time for reconstitution. In order to meet the requirement of Armed Forces, instant vegetable *pulav* mix based on rice, potatoes and peas capable of reconstitution by mere mixing with hot water has been developed. The present paper describes optimisation of the process for preparation of easy-to-cook vegetable *pulav* mix and evaluate its storage stability in flexible packs.

Materials and Methods

Good quality 'Bangaratheega' (BT) rice (*Oriza sativa*), potatoes (*Solanum tuberosum*), fresh green peas (*Pisum sativum*) and whole spices were procured from the local market, manually cleaned to remove dirt, dust and foreign materials. Vanaspati (Dalda) and table salt were also purchased from the local market.

Preparation of freeze-thaw dehydrated rice : Good quality BT rice (10 kg) was washed with water to remove adhering starch. The washed rice was cooked with 25 litres of water in an autoclave at 1.08 kg/cm² steam pressure for 15 min and cooled to room temperature. Cooked rice was spread in trays and frozen in a blast freezer (Model 15L-U2-300, M/s Hull Corporation Hatboro, USA) by keeping at -20°C for 8-10 hrs till all grains got frozen. Frozen rice was then allowed to thaw at room temperature and subsequently, transferred to a fluidised bed dryer (Model 30 D, Chemec Eng, Mumbai, India) and dried at 70°C to a moisture level of about 5%.

Frying of green peas : Green peas (5 kg) were depoded and blanched in boiling water containing 1% sodium carbonate for 5 min. The blanched peas were allowed to cool and subsequently, fried in 500 g lots in 2 kg vanaspati at 140°C for 5-10 min, till most of the moisture was removed. The fried green peas were then dried in a fluidised bed dryer at 70°C to a final moisture of about 5%.

Frying of potatoes : Good quality potatoes (5 kg) were peeled in an abrasion type mechanical peeler (M/s Gardner's, New Delhi, India). The peeled potatoes were cut into 1 × 1 × 4 cm size fingers in a semi-automatic potato chipper (Crystal, M/s Kitchenwares, Rajkot, India) in an aqueous solution containing potassium metabisulphite (500 ppm). The finger chips were fried in 250 g lots in vanaspati at 160°C for 15-20 min. The fried potato fingers were finally dried in a fluidised bed dryer at 70°C to a moisture of about 5%.

Preparation of spice mix : The whole spices were separately powdered in an ultracentrifugal mill (Retsch R1, Germany) using 1 mm sieve. Spice mix consisted of star-anise (*Illicium verum*) powder (5 g), clove (*Eugenia caryophyllus*) powder (2 g), Cinnamon (*Cinnamomum zeylanicum*) powder (3 g), black cardamom (*Amomum subulatum*) powder (3 g), Cardamom (*Elettaria cardamomum*) powder (2 g), red chilli (*Capsicum annum*) powder (62 g), turmeric (*Curcuma longa*) powder (16 g), tejpat (*Cinnamomum tamala*) (1 g) and citric acid (6 g), stirred in a Warning blender to get a homogeneous product.

Blending : Vanaspati (750 g) was heated in a stainless steel vessel to 120°C and whole cloves (30 g), black pepper (30 g) and tejpat leaves (15 g) were added and heated for 2 min. The spice mix (225 g) was then added to hot vanaspati with continuous mixing. The vessel was removed from the flame and instant rice (5 kg), fried peas (1.5 kg), fried potatoes (1.25 kg) and salt (225 g) were added in the hot spice mix and strirred continuously to ensure uniform coating of the spices.

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Packaging and storage : Instant vegetable *pulav* mix (200 g) was packed in polypropylene (PP, 75μ) and paper (42 GSM)-aluminium foil (20 μ) polyethylene (37.5μ) laminate (PFP) pouches (15 X 15 cm) and hermetically sealed. Fifteen pouches of each were stored at 0°C, room temperature (15-34°C) and 37°C, respectively. Initially and after every three months, two samples of each were removed and analysed for chemical, reconstitution and sensory characteristics.

Analysis : Moisture, proteins, fat, total ash and chlorophyll were determined by standard AOAC (1984) methods. Storage changes in instant vegetable *pulav* were monitored by determining peroxide value (PV) and free fatty acids (FFA) as per AOCS (1973) methods, while thiobarbituric acid value (TBA) and total carotenoids were determined as per the methods of Tarledgis et al (1960) and Sharma et al (1996). Mineral composition of instant vegetable *pulav* mix was determined by atomic absorption spectrophotometer (model AA 670, Shimadzu, Kyoto, Japan) as per the method of Semwal et al (1995).

Adsorption-desorption characteristics : Powdered samples (100 g) of instant vegetable *pulav* mix were stored for 30 days in petri dishes in desiccators containing phosphorus pentoxide and saturated solution of magnesium chloride, sodium bromide and sodium nitrate to obtain water activity (a_w) of 0.0, 0.33, 0.57 and 0.73, respectively. Initially and periodically, the stored samples were analysed for peroxide value, free fatty acids and moisture content.

Reconstitution and sensory evaluation : Instant vegetable *pulav* mix (100 g) was treated with 300 ml hot water (90°C) in a stainless steel vessel, covered and set aside. After 10 min, the samples were mixed with spoon and presented to a panel of 15 trained judges for grading for colour, taste, flavour and overall acceptability on a 9-point Hedonic scale with 9 for excellent in all respects and 1 for highly disliked samples.

Results and Discussion

Instant vegetable *pulav* mix procesed by the modified method got reconstituted by mere mixing with hot water and thus provided a lot of convenience over the previously developed pre-cooked dehydrated vegetable *pulav*. The flow chart for the preparation of instant vegetable *pulav* mix is given in Fig. 1. The proximate and mineral composition of instant vegetable *pulav* mix is given in Table 1. Based on the proximate composition, its total energy value works out to be 460 kcal/100 g and therefore it is sufficiently calorie dense and highly suitable for operational pack rations. A minimum shelf life of one year under all weather conditions is an essential requirement laid down by Indian Armed Forces for all operational pack rations (Semwal and Arya 1992) and therefore, shelf life of instant vegetable *pulav* mix packed in polypropylene (PP) and paper-aluminium foil-polyethylene (PFP) laminate pouches was evaluated. Initially, the instant vegetable *pulav* mix had an overall acceptability score of 8.4 on a 9-point Hedonic scale and therefore, a score of 7.0 was taken as a limit of shelf life in storage experiments. Because of low moisture content in instant vegetable *pulav*, off-flavour emanating from oxidative degradation of lipids and carotenoids

TABLE 1. PROXIMATE AND MINERAL COMPOSITION OF INSTANT VEGETABLE *PULAV* MIX

Moisture, %	5.1 ± 0.10
Proteins, %	7.3 ± 0.20
Fat, %	19.8 ± 0.30
Total ash, %	5.2 ± 0.20
Carbohydrates, %	62.6 ± 0.40
Sodium, mg/kg	19,200 ± 64
Potassium, mg/kg	5160 ± 22
Calcium, mg/kg	725 ± 18
Iron, mg/kg	85 ± 70
Zinc, mg/kg	26 ± 2.50
Copper, mg/kg	3.8 ± 0.40

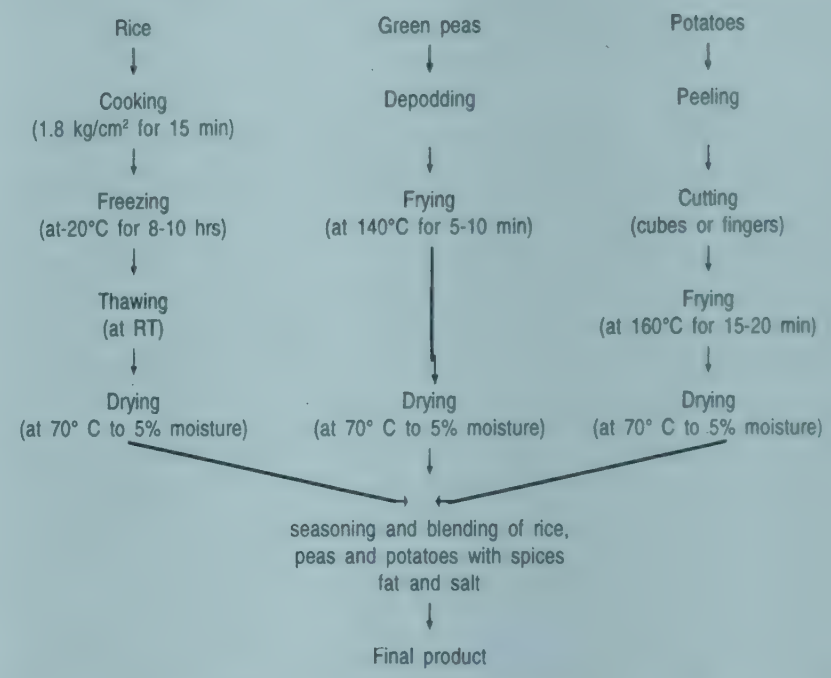

Fig. 1. Process flow chart for making instant vegetable *pulav* mix

is the major cause of reduced acceptability of *pulav* during storage. The changes in PV, FFA, TBA and overall acceptabilty scores of instant vegetable *pulav,* when stored at 0°C, room temperature and 37°C in PP and PFP pouches are given in Table 2. It was observed that the rates of autooxidative deterioration were higher in samples stored in PP as compared to PFP stored samples. Both PV and TBA increased during storage at all the three temperatures (0, RT and 37°C). The rate of increase was however, slightly lower at 0°C. After 12 months of storage, PV increased from 2.0 to 4.6 and 5.7 at 0°C, 7.2 and 9.8 at room temperature and 8.5 and 11.6 meq O_2/kg fat at 37°CC in PFP and PP pouches, respectively. The TBA value also followed a similar pattern and ranged from 0.05 to 0.29 mg MA/kg throughout the storage. FFA in instant vegetable *pulav* mix in both the packaging materials also increased on storage and at all the three temperatures. After 12 months of storage, FFA value increased from 0.08 to 0.20 and 0.22 at 0°C, 0.30 and 0.31 at RT and 0.42 and 0.57% oleic acid at 37°C in PFP and PP, respectively.

During storage of instant vegetable *pulav*, concentration of total carotenoids and chlorophyll decreased considerably, affecting the acceptability of the product. The changes in total

TABLE 2. CHANGES IN PEROXIDE VALUE (PV), FREE FATTY ACIDS (FFA) AND THIOBARBITURIC ACID VALUE (TBA) IN INSTANT VEGETABLE *PULAV* MIX STORED IN POLYPROPYLENE (PP) AND PAPER-ALUMINIUM FOIL-POLYETHYLENE (PFP) LAMINATE POUCHES AT 0°C, ROOM TEMPERATURE (RT) AND 37°C

Storage period, months	Storage temperature, °C	PV		FFA		TBA	
		PFP	PP	PFP	PP	PFP	PP
0	-	2.0	-	0.08	-	0.05	-
3	0	2.8	3.9	0.10	0.11	0.07	0.08
	RT	3.6	5.5	0.10	0.13	0.09	0.12
	37	4.0	6.8	0.11	0.13	0.14	0.16
6	0	4.1	4.9	0.11	0.12	0.11	0.13
	RT	5.4	7.8	0.11	0.15	0.14	0.15
	37	5.9	8.9	0.17	0.19	0.17	0.20
9	0	4.3	5.2	0.15	0.18	0.12	0.13
	RT	6.1	8.9	0.19	0.20	0.16	0.18
	37	6.9	10.1	0.29	0.32	0.20	0.25
12	0	4.6	5.7	0.20	0.22	0.12	0.14
	RT	7.2	9.8	0.30	0.31	0.18	0.21
	37	8.5	11.6	0.42	0.57	0.23	0.29

All results are mean of two values

TABLE 3. CHANGES IN TOTAL CAROTENOIDS (µg/g), CHLOROPHYLL (mg/100g) AND OVERALL ACCEPTABILITY (OAA) SCORE OF INSTANT VEGETABLE *PULAV* STORED IN POLYPROPYLENE (PP) AND PAPER-ALUMINIUM FOIL-POLYETHYLENE (PFP) LAMINATE POUCHES AT 0°C, ROOM TEMPERATURE (RT) AND 37°C

Storage period, months	Storage tempera-ture, °C	Total carotenoids		Chlorophyll		Overall acceptability*	
		PFP	PP	PFP	PP	PFP	PP
0	-	18.8	-	4.8	-	8.4 ± 0.2	-
3	0	18.5	18.1	4.7	4.6	8.2 ± 0.1	8.0 ± 0.3
	RT	18.3	18.0	4.4	4.2	8.0 ± 0.3	7.7 ± 0.2
	37	17.4	16.8	4.2	4.0	7.8 ± 0.2	7.5 ± 0.1
6	0	18.2	17.8	4.6	4.2	8.0 ± 0.4	7.7 ± 0.2
	RT	17.7	16.9	3.8	3.4	7.8 ± 0.2	7.5 ± 0.1
	37	16.4	15.7	3.6	3.1	7.4 ± 0.1	7.1 ± 0.1
9	0	17.9	17.7	4.6	4.1	7.8 ± 0.3	7.7 ± 0.2
	RT	16.7	15.3	3.6	3.0	7.6 ± 0.2	7.1 ± 0.1
	37	14.5	13.2	3.2	2.8	7.1 ± 0.1	6.5 ± 0.2
12	0	17.5	17.2	4.2	4.0	7.7 ± 0.4	7.5 ± 0.3
	RT	14.6	12.6	3.5	2.9	7.2 ± 0.1	6.6 ± 0.2
	37	11.3	8.9	3.0	2.4	6.7 ± 0.1	6.0 ± 0.1

All results are mean of two values
* Mean ± SD (n = 15)

carotenoids and chlorophyll contents were measured during storage and results are given in Table 3. Changes in total carotenoids and chlorophyll seemed to follow as those of overall acceptability scores. After 12 months of storage, total carotenoids decreased by 8.5 and 6.9% at 0°C, 32.9 and 22.3% at RT and 52.7 and 39.9% at 37°C. Also, decreases were higher in PP than in PFP packs at all the three temperatures. Similarly, total chlorophylls decreased by 16.7 and 12.5% at 0°C, 39.6 and 27.1% at RT and 50.0 and 37.5% at 37°C after 12 months in PP and PFP, respectively. Earlier also, Sharma et al (1995) concluded that the carotenoids degraded faster in PP than in PFP packs and the rate of degradation increased with increasing storage temperature.

Effect of water activity (a_w) : Sorption isotherm of instant vegetable *pulav* mix and the changes in PV and FFA in instant vegetable *pulav* mix, when stored at different a_w for 30 days are shown in Fig. 2. Instant vegetable *pulav* mix equilibrated to 2.6, 5.9, 8.2 and 12.2 % moisture at 0.0, 0.33, 0.57 and 0.73 a_w respectively. No microbial spoilage was observed, when instant vegetable *pulav* mix was stored up to 0.73 a_w for 30 days at room temperature (15-34°C). However, PV and FFA increased at all a_w during storage. The rate of increase was lowest at 0.33 a_w and the rate of formation of PV and FFA increased considerably both below and above 0.33 a_w, indicating maximum product stability around 0.33 a_w. This is in conformity with the published data, as water forms hydrogen bonds with hydroperoxides and thus prevents their undesirable decomposition at lower a_w (Labuza 1978). Water also hydrates transition metal ions, which are known to catalyse autooxidation of fats (Labuza 1978). Normally, increase in FFA content in stored products results from the lipolysis of lipids. However, in instant vegetable *pulav,* lipolytic enzymes get inactivated during cooking, frying and drying operations and increase in FFA occurs as a result from the hydrolysis of triglycerides in the presence of water.

It is evident that highly acceptable instant vegetable *pulav* mix, capable of reconstitution by mere mixing with hot water, can be prepared by freeze-thaw dehydrated rice and

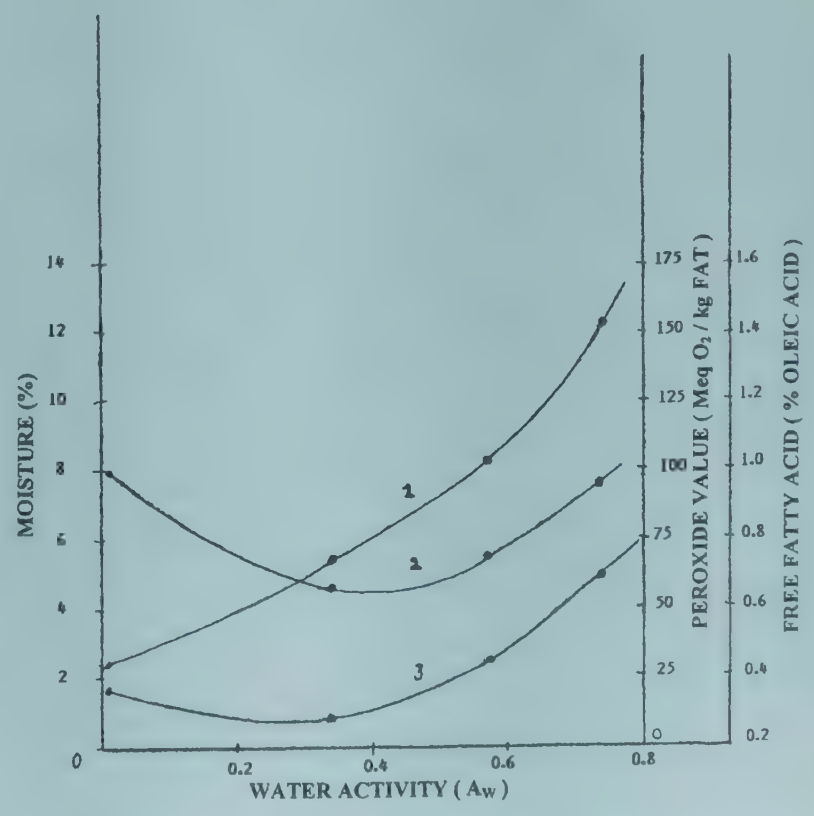

Fig. 2. Effect of water activity on moisture (1), peroxide value (PV, 2) and free fatty acid (FFA, 3) contents of instant vegetable *pulav* mix after 30 days of storage at room temperature (15-34°C)

blending with fried potatoes, fried green peas, spices and vanaspati. Autooxidation of lipids and carotenoids and loss in flavours are the major causes of storage deterioration. Instant vegetable *pulav* mix remained stable and acceptable for 12 and 9 months at RT and 37°C, when packed in PFP and also had a useful shelf life of 9 and 6 months at RT and 37°C in PP packs.

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RESEARCH PAPER J. Food Sci. Technol., 2001, Vol. 38, No. 3, 235-238

Efficacy of Casein Coating on Storage Behaviour of Kinnow

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The effect of storage under ambient conditions of kinnows coated with casein film and packed in low and high density polyethylene bags on some quality parameters was studied. The quality parameters such as physiological weight loss, firmness, peel thickness, juice and the decay were measured. It was found that the casein film delayed the loss of firmness, with minimum loss in physiological weight and juice level during storage. It was possible to extend the shelf-life of kinnows by 20 days without any significant change in quality with the use of this technique.

Keywords : Kinnow, Storage, Low density polyethylene, High density polyethylene, Casein, Edible coating.

Several types of protein-based film coatings have been applied successfully by several researchers for preservation of fresh products (Andres 1984; Park et al. 1994; Lerdthangkul and Krochta 1996). The films were found to be very efficient oxygen barrier but had high water vapour permeability compared to plastic films (Avena and Krochta 1993; Park and Chinnan 1995). Modification of internal gas composition by edible coatings can cause disorders due to high CO_2 and low O_2 levels (Ben-Yehoshua 1985; Smith et al. 1987). Several problems such as anaerobic fermentation have been associated with edible coatings of apples and bananas (Banks 1984; Smock 1940).

The effectiveness of edible coatings for fruits and vegetables depends primarily on selection of appropriate films or coatings, which result in beneficial internal gas composition. Coatings on fruits and vegetables that exceed critical thickness can cause detrimental effects by reducing internal O_2 and increasing CO_2 concentration leading to anaerobic fermentation. This paper reports the findings of a study undertaken to evaluate the effects of storing kinnow fruits coated with casein film and packed in LDPE and HDPE bags on some quality parameters.

Materials and Methods

Preparation and coating of kinnows : Aqueous solution of 8% (w/w) casein formulation (Habig Mchugh et al. 1993) was prepared by adding required amount of casein alkali soluble (CAS) powder to 184 ml of 0.1 N NaOH at room temperature (18°C) in a 500 ml flask. The solution was warmed to 50°C on a hot plate magnetic stirrer for 30 min and then cooled to room temperature. Glycerol was added as a plasticizer equal to 50 % of the original weight of CAS and subjected to vaccum to prevent formation of air bubbles in the film prior to use. To stabilize the protein coating (CAS) formed on the fruit, it was treated with sodium acetate buffer (pH 4.6) by dipping the fruit for one minute (Avena Bustillos and Krochta 1993). The flow diagram for the technique used is presented in Fig. 1.

Storage : Kinnows coated with casein films packed in low and high density polyethylene bags (200 guage) were stored at 18 to 28°C temperature and 55 to 65% relative humidity for 10, 20 and 30 days. Each bag (sample) contained three fruits. All the treatments were replicated thrice. The data were statistically analysed using factorial experiment in Completely Randomised Design by using computer software package (Cheema and Singh 1990). The least significant difference (LSD) was calculated at 5% level of significance (p = 0.05).

Quality parameters : Initial weight of each sample was recorded at the time of keeping the sample. The cumulative percent loss (physiological loss) in weight was calculated on fresh weight basis. The firmness, which indicated the hardness or softness of fruit was measured with the help of a fruit tester or penetrometer (McCormick Fruit Tester FT-527). The fruit tester measures the firmness in the range of 1.5 to 12 Kg-f. The individual fruit was put to a pressure so that the probe of penetrometer panetrated the fruit. The observations were taken around the circumference of fruits at three different positions and final reading was expressed as an average of three,

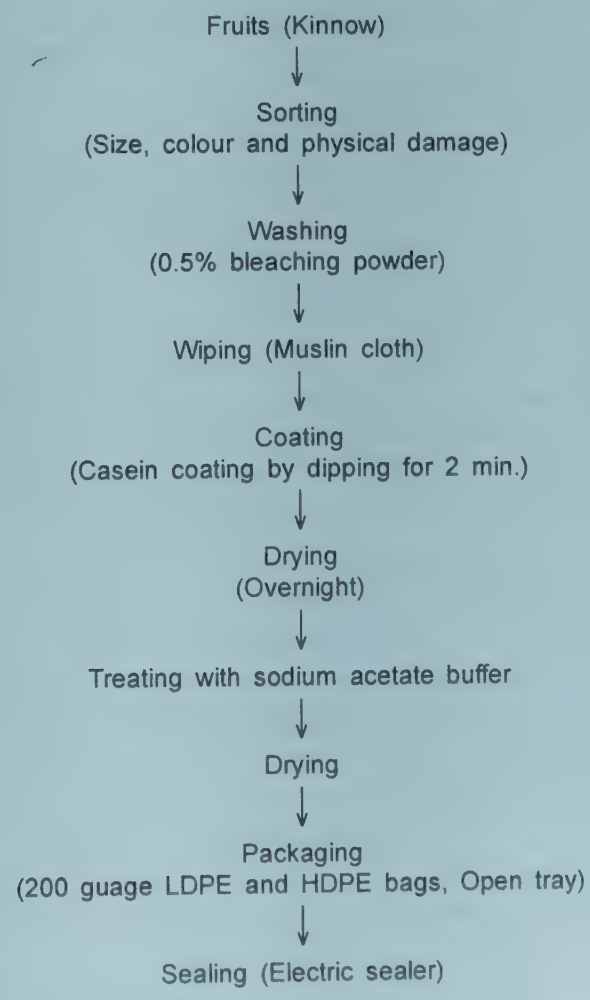

Fig. 1. Process flow diagram for coating of kinnows

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TABLE 1. EFFECT OF COATING, PACKAGING MATERIAL AND STORAGE ON QUALITY PARAMETERS

Quality parameter	Coatings (A)		Packaging (B)			Storage period, days (C)			
	Control	Casein	Tray	LDPE	HDPE	0	10	20	30
PWL, %	5.97	4.70	14.39	0.88	0.74	0.00	4.10	6.87	10.38
LSD	0.374			0.458			0.529		
(p= 0.05)	AB: 0.649		AC: 0.749		BC : 0.917		ABC: 1.298		
Firmness, Kg-f	5.67	6.28	6.36	5.69	5.89	6.25	6.46	5.93	5.27
LSD	0.025				0.031		0.036		
(p= 0.05)	AB: 0.044		AC: 0.051		BC : 0.063		ABC : 0.088		
Peel thickness, mm	2.78	2.92	2.31	3.08	3.16	3.52	2.87	2.59	2.42
LSD	0.005				0.006		0.007		
(p= 0.05)	AB: 0.008		AC: 0.007		BC : 0.001		ABC: 0.002		
Juice content, %	36.35	37.92	40.54	35.27	35.60	38.16	37.72	37.38	35.28
LSD	1.064			1.303			1.504		
(p= 0.05)	AB: NS		AC: NS		BC : 2.605		ABC: 3.685		
Decay, %	12.02	11.88	9.31	13.46	13.09	0	4.48	10.84	32.49
LSD	NS			0.862			0.995		
(p= 0.05)	AB: 1.219		AC: 1.408		BC : 1.724		ABC: 2.439		

AB, AC, BC, ABC represent the interaction of different treatments

Kg-f. The average peel thickness of the fruits was recorded with the help of a micrometer (The least count of the micrometer was 0.002 mm). The juice was extracted from two fruits with the help of a manual juice extractor, strained through muslin cloth and the volume occupied was measured by a graduated cylinder. The percent juice recovery was calculated on the basis of fresh fruit weight before extracting the juice. The decay, expressed as total percent incidence of both rotten and fungal infected fruits, was calculated by counting the fruits that were found rotten on visual observation after each storage interval.

Results and Discussion

Weight loss : Different treatments on kinnow fruits resulted in significant ($p = 0.05$) losses in weights during storage (Table 1). The coating of kinnows with casein film resulted in less weight losses irrespective of packaging material during storage (Fig. 2). A minimum weight loss (1.5%) occurred in fruits coated with casein and packed in HDPE bags and the maximum weight loss (33.23%) was found in non-coated fruits kept in open tray, after 30 days of storage. Similar effects of the wax coating and sucrose esters on weight losses of oranges, bananas and apples during storage have been reported (Ben-Yehoshua 1967; Banks 1984; Smith and Stow 1984).

Firmness: The casein coating delayed the loss in firmness of kinnows. Firmness of casein-coated fruits showed initial increase followed by a decrease of 10.4% for the fruits packed in high density polyethylene bags after 30 days of storage, while the firmness of casein-coated kinnows without any packaging material remained without changes during storage (Fig. 3). The initial increase might be due to the increased resistance to diffusion of carbondioxide (CO_2) and oxygen (O_2) resulting in elevated CO_2, reduced O_2 and concomitantly, reduction in respiration rate. The decrease in firmness in the later period could be attributed to degradation of cellulose in the cell wall (John et al. 1989). Casein-coated fruits kept in open tray maintained the firmness, while firmness of the control fruits packed in polyethylene bags significantly decreased ($p = 0.05$) (Table 1).

Peel thickness : Peel thickness of kinnows decreased during storage irrespective of the packaging material (Fig. 4). Coating, packaging material, storage period and their interactions significantly ($p = 0.05$) affected the peel thickness (Table 1). The

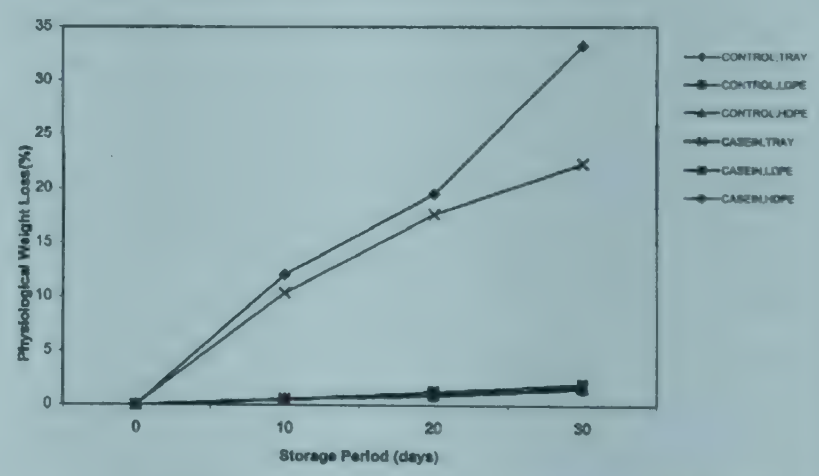

Fig. 2. Effect of coating, packaging material and storage period on physiological weight loss

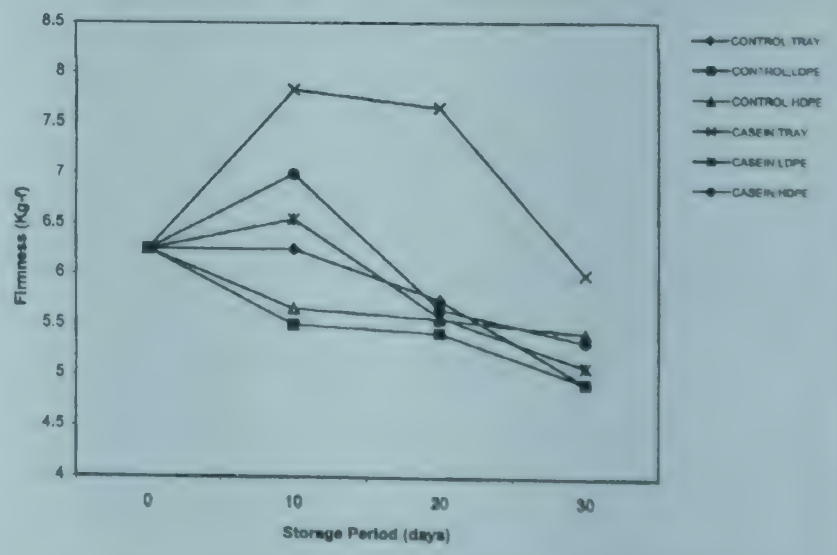

Fig. 3. Effect of coating, packaging material and storage period on firmness

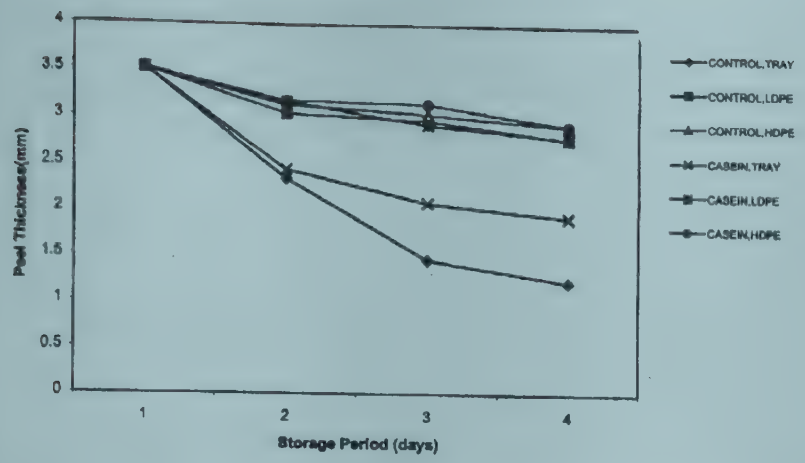

Fig. 4. Effect of coating, packaging material and storage period on peel thickness

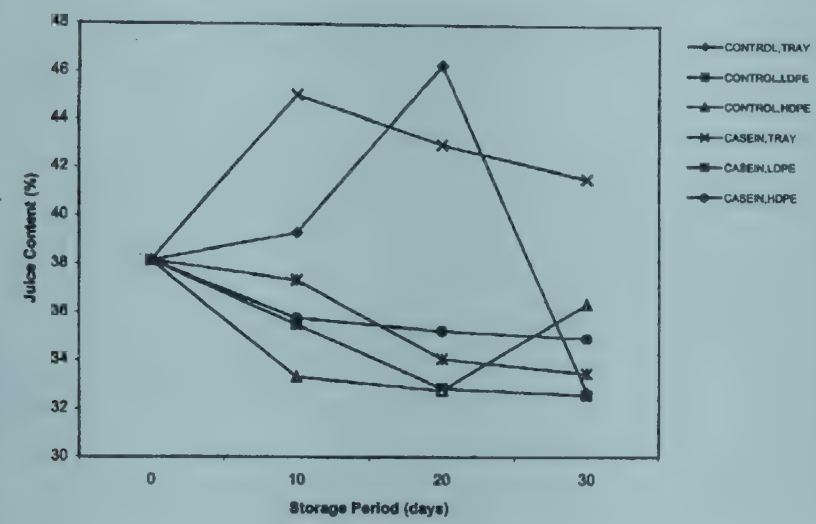

Fig. 5. Effect of coating, packaging material and storage period on juice content

fruits coated and stored in polyethelyne bags showed higher peel thickness as compared to those kept in open tray. The minimum reduction of 10.7% in peel thickness was noticed in casein-coated fruits kept in HDPE bags. This may be due to the reduced psysiological water loss, resulting in less shrinkage and softening of the peel. Sealed packaging is also known to markedly increase the healing of superficial scratches of mechanically harvested fruits and induce the activity of phenyl alanine ammonia lyase (PAL) enzyme, which helps in formation of lignin precursors, thereby maintaining the peel thickness during storage (Ben Yehoshua 1967).

Juice recovery : Different treatments have resulted in significant variations ($p = 0.05$) in juice content of kinnows, while the interactions (coating, packaging material and coating, storage period) showed statistically no significant affect on juice content (Table 1). The coated fruits kept in open tray showed a slight initial increased, followed by steady decrease in the juice content compared to polyethylene-sealed fruits (Fig. 5). This might be due to the fact that there was an initial loss of moisture from the peel, while the juice was obtained from the pulp (El. Zeftawi 1976); whereas, the significant decrease in juice percentage after prolonged storage was probably due to the continuous dehydration of peel and juice (Das and Dash 1967). Casein-coated fruits showed a steady level of juice percentage irrespective of packaging material when compared to non-coated fruits.

TABLE 2. EFFECT OF COATING, PACKAGING MATERIAL AND STORAGE PERIOD ON DECAY PERCENTAGE

Treatments	Storage period, days			
	0	10	20	30
Control, Tray	0	6.35	15.87	25.39
Casein, Tray	0	0.00	4.76	22.22*
Control, LDPE	0	3.17	7.93	39.68
Casein, LDPE	0	7.94	15.87	38.09
Control, HDPE	0	1.59	6.35	38.09
Casein, HDPE	0	7.94	14.28	36.51

*Minimum loss after 30 days of storage

Decay : The packaging material and storage period significantly ($p = 0.05$) increased the decay percentage, while the coating showed statistically no significant effect on decay (Table 1). The casein-coated fruits packed in polyethylene bags were more prone to the fungal decay after prolonged storage (Table 2). This increase in decay might be due to the increase in CO_2 percentage in the polyethylene bags, and also, due to the soilage, that is, the contamination of adjacent fruits by sporulation and drip from the rotten one (Ben-Yehoshua et al. 1981). The minimum decay (22.22%) was noticed in casein-coated fruits kept in open tray and maximum decay was observed in casein-coated fruits (38.09%) kept in low density polyethylene bags.

Conclusions

The storage of kinnows coated with casein film under ambinet conditions delayed the loss in firmness and maintained high juice percentage with minimum decay, while those coated with casein film and packed in HDPE bags showed the lowest reduction only in physiological weight loss and peel thickness. The use of casein film coating without any packaging material can enhance the shelf life of kinnows by 20 days without any significant change in quality.

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RESEARCH PAPER

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Effect of Conventional Versus Ultrapasteurization of Egg Components on the Quality of Custards and Cakes

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Effect of ultrapasteurization of egg components on the quality of custards and cakes was studied. Ultrapasteurization caused significant ($P<0.05$) increases in coagulation content and pH of liquid white, while significant decreases were observed in percent moisture absorption, foam draining characteristics, cake volume and cake height. Cakes prepared from ultrapasteurized samples were poor in shape, when compared to those by conventional pasteurization. However, organoleptic evaluation scores showed slight improvement in ultrapasteurized sponge cake made of yolk. Baked custard did not show any significant difference between two treatments in respect of organoleptic qualities.

Keywords : Egg components, Custards, Cakes, pH, Cake volume, Cake height, Percent moisture absorption, Shape characteristics, Organoleptic qualities.

In India, annual production of shell eggs has increased from 22,000 millions in 1990 to 35,000 millions in 1996 and processed egg products from 28,050 in 1993-94 to 32,596 metric tonnes in 1995-96. In addition, the country exports shell egg and dehydrated products. The presence of *Salmonella enteritidis* in egg and egg products has caused a dramatic decrease in the sale of these products in several countries of the world. Fresh shell egg consumption is likely to drop further from 94, as it was during 1990 to 90% in 2000 and processed egg products would increase from 6% in the same year to 10% in 2000 (Panda 1997). Losses due to microorganisms esp., *Salmonella senftenberg* 775W caused a great concern to scientists, who had to attempt new technologies viz., hyper-pasteurization, ultrapasteurization etc. for improving the safety of the product, in spite of the conventional pasteurization being successfully used till now. Keeping these in view, the present study was undertaken to study the effect of ultrapsteurization of egg components on the quality of custards and cakes as compared to conventional pasteurization.

Materials and Methods

Collection of sample : Fresh eggs (less than two days old) were collected in batches of hundreds from the College Poultry Farm, maintaining White Leghorn birds. All the eggs used in the study were from the same breed, same age and managed under similar conditions of feeding and management schedule in the same season.

Preparation of sample : Immediately after collection, the eggs were transferred to the laboratory for candling. Only clean sound shelled eggs without any defects were used in the study. Ninety eggs were selected and divided into three batches of 30 each for three trials. These 30 eggs were further divided into batches A and B of 15 each. Batch A was used for collection of whole egg liquid (WEL) and batch B for separation of white and yolk. The eggs were dipped in ethanol, air-dried, and broken into different sterile glass beakers for WEL, yolk and liquid white separately and blended by placing in a sterile blender.

Pasteurization technique : One half portions of each of WEL, yolk and liquid white in beakers were pasteurized by placing at constant temperature water bath at 60°C for 3.5 min (conventional) and the other half at 70°C for 60 sec. (ultrapasteurization); 61.1°C for 3.5 min (conventional) and 68°C for 98 sec. (ultrapasteurization); 57.7°C for 3.5 min (conventional) and 68°C for 60 sec. (ultrapasteurization) respectively.

After pasteurization, the containers were transferred to an ice water bath, till the temperature of the contents in the beaker reached 1.1°C. At the end of pasteurization, amylase activity was estimated to ascertain adequacy of pasteurization by following the method of Imai (1979).

Functional properties : Eggs are usually incorporated in food products due to their ability to coagulate, foam and emulsify. Performance testing of these functional properties on the quality of custards and cakes was done by standard procedure as indicated below :

Basic custard recipe consisted of 237 ml milk, 48 g egg, 25 g sugar, 0.5 g salt and 1.2 ml vanilla.

Stirred custard : Milk was scalded over boiling water. Eggs, sugar and salt were beaten, while stirring constantly and half of the scalded milk was added to it slowly and the next half rapidly. When the mixture was homogenously set, it was cooked over simmering water, ensuring that it does not boil. The contents were constantly stirred until the mixture coated the spoon. Vanilla was then added. The product was removed from the heat source and poured into the small custard containers and chilled at 4.4°C for 18 h.

Bakèd custard : The oven was set at a pre-heating temperature (80°C). Milk was scalded in boiling water and a mixture of salt, sugar, vanilla was prepared. Scalded milk was slowly added first and then rapidly with constant stirring. Then, the complete custard mixture was transferred to custard cups. Cups were then transferred to a thermostatically controlled oven at 180°C. Custard cups were removed from the oven, when the internal temperature came to 86-87°C and then chilled in a refrigerator at 4.4°C for 18 h. Organoleptic evaluation of custards was done by a panel of 6 experienced judges

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following a 10-point score card for colour, texture, flavour and overall acceptability (Hanson et al. 1947).

Chilled stirred custards were then tested for coagulation property by Line Spread Test. Line Spread Test required a levelled glass plate, consisting of a series of numbered concentric circles spaced at 1/8 inch apart (15 in number), the diameter of the innermost circle being 2 inch. A hollow glass cylinder 2 inch in diameter and 1 inch in height was then placed over the centre of the concentric circles and was filled with custard. The cylinder was then quickly removed allowing the custard to spread for 2 min. The extent of spread was noted at 4 widely separated points by reading the number of lines through which the custard moved. The number of the line starting from the second circle (taken as number one) was recorded. Parallel to it, custards prepared from unpasteurized eggs stored at the same room temperature and humidity schedule were also evaluated for coagulation (Panda 1994). Baked custards were evaluated for percentage of sag, which was calculated based on the depth of the custard at two periods (Krammer and Twigg 1973).

Foaming properties of the egg contents were evaluated by performance of the Angel cake by following the method of Slosberg et al (1948).

Cake volume and cake height : Cake volume was estimated by Seed Displacement Method. The surface above the cake was filled with seed and levelled off with a spatula. Seeds were then poured into a graduated cylinder and volume was recorded. The volume of cake pan minus the volume of seeds was considered as the volume of cake. Cake height was measured after cutting the cake in the center using a scale calibrated in centimeters.

Textural properties were also determined by ink print method. Cakes were cut near the center and were pressed over the ink pad evenly and firmly. Then, the same was printed over a graph paper to obtain the cake texture. Moisture absorption test on cake was done as per the method of Kramer and Twigg (1973).

Direct foaming test : Albumen (100 ml) was beaten for 90 sec each at medium and at high speed with the aid of an electric mixer, smoothened out the top of the foam with a rubber spatula and a ruler was inserted into the beaker to note the depth of the foam. Foam was then transferred to large funnel with the aid of a spatula to collect the separating liquid in a graduated cylinder. The volume of the liquid collected in the cylinder after one hour was noted. This was done to assess the stability of the foam (Panda 1994). Properties of liquid whole egg and yolk were judged on the performance in preparation of sponge cakes by following the method suggested by Kramer and Twigg (1973). The cake volume, cake height, moisture absorption and organoleptic characteristics for tenderness, texture, colour and flavour were determined as described earlier.

Statistical analysis : Data thus collected were subjected to analysis of variance (ANOVA), Duncan's multiple range test (DMRT) and student's t-test to compare any significant difference among the mean values (Snedecor and Cochran 1967).

Results and Discussion

Functional properties of liquid white : The drainage of foam (ml) observed under conventional pasteurization (9.0) was significantly higher compared to ultrapasteurization (8.8) (Table 1). This was consistent with earlier findings of Miller and Winter (1950), Garibaldi et al (1969) and Stadelman and Cotterill (1977). Perhaps, this was due to the adverse effect of heat on the foaming properties of egg white. Garibaldi et al (1969) found that ovomucin lysozyme network gets denatured irreversibly due to heat. Johnson and Zabik (1981) have already reported that the interaction of lysozyme and globulin, which was an important function for the foaming process, undergoes destruction during thermal process. Cunningham (1995) had also stated that the decline in foaming properties became evident, when the albumen was subjected to a temperature of 57°C and above.

In conventional pasteurization, pH of liquid white was significantly ($P<0.05$) lower than that in ultrapasteurization (Table 1). This was similar to observations made by Ball et al (1987) and Martinez et al (1995). This could be due to breakage of peptide linkages of alkaline amino acid during the process of ultrapasteurization.

A significant ($P<0.05$) decrease in cake volume (380.4 ml) and cake height (7.9 cm) was observed, when the liquid white was ultrapasteurized (Table 1). Hanson et al (1947) observed that heating of egg white beyond 58.8°C impaired cake volume. Bergquist (1977) indicated that heating of egg white at 54°C for 3 min resulted in a decrease in Angel cake volume. Wong et al (1996) were of the opinion that pasteurization reduced the cake volume. Clinger et al (1950) showed significant decrease in cake height following pasteurization, while Martinez et al (1995) found that the temperature had a greater effect on cake height than holding time. Protein content did not differ between

TABLE 1. EFFECT OF CONVENTIONAL VERSUS ULTRAPASTEURIZATION ON FUNCTIONAL PROPERTIES OF LIQUID WHITE

Functional properties	Pasteurization techniques	
	Conventional	Ultrapasteurization
Foaming property (Drainage/90 sec) ml	$9.0^{b} \pm 0.04$	$8.8^{a} \pm 0.01$
pH of Angel cake	$8.2^{a} \pm 0.01$	$8.7^{b} \pm 0.01$
Angel cake height, cm	$8.5^{b} \pm 0.06$	$7.9^{a} \pm 0.06$
Angel cake volume, ml	$410.9^{b} \pm 3.10$	$380.4^{a} \pm 7.85$
Protein content, %	$8.7^{a} \pm 0.03$	$8.5^{a} \pm 0.23$

Each value with different superscripts in a row are different significantly ($P<0.05$). Each value is a mean ± SE of three experiments

TABLE 2. EFFECT OF CONVENTIONAL VERSUS ULTRAPASTEURIZATION OF WHOLE EGG LIQUID ON SPONGE CAKE CHARACTERISTICS

Characteristics	Conventional pasteurization	Ultra pasteurization	Actual difference	Percent difference
Cake volume, ml	673.0	661.7	11.3	1.67
Cake height, cm	6.0	5.9	0.1	2.79

Each value is a mean of three experiments

the two treatments. This was in confirmity with the findings of Ball et al (1987) and Martinez et al (1995).

Functional properties of whole egg liquid : Results pertaining to sponge cake volume containing WEL indicated more reduction in ultrapsteurized samples when compared with conventional ones (Table 2). This was in confirmity with the results of Hanson et al(1947), Winter (1952) and Sugihara et al(1966).

TABLE 3. EFFECT OF CONVENTIONAL VERSUS ULTRAPASTEURIZATION OF LIQUID WHITE AND WHOLE EGG LIQUID ON SHAPE CHARACTERISTICS OF CAKES

Treatments	Shape characteristics, % Sag	Flat	Round
Liquid white (Angel cakes)			
Conventional pasteurization	2.0[a]	15.0[a]	83.0[b]
Ultrapasteurization	12.66[b]	23.66[b]	63.66[a]
t-value at 5% level	10.13	5.97	12.38
Whole egg liquid (Sponge cakes)			
Conventional pasteurization	0.6[a]	18.0[a]	81.33[b]
Ultrapasteurization	14.66[b]	24.0[b]	61.33[a]
t-value at 5% level	9.37	4.65	9.74

Each value is a mean of three experiments

Figures with different superscripts in each product in a column differ significantly (P<0.05)

TABLE 4. EFFECT OF CONVENTIONAL VERSUS ULTRAPASTEURIZATION OF WHOLE EGG LIQUID AND LIQUID WHITE ON COAGULATION PROPERTY OF CUSTARDS

Treatments	Whole egg liquid, cm	Liquid white, cm
Conventional pasteurization	0.95[b] ± 0.003	0.83[b] ± 0.003
Ultrapasteurization	0.81[a] ± 0.003	0.78[a] ± 0.003

Figures with different superscripts in a column differ significantly (P<0.05)
Each value is a mean ± SE of three experiments

TABLE 5. EFFECT OF CONVENTIONAL VERSUS ULTRAPASTEURIZATION ON THE ORGANOLEPTIC QUALITY OF BAKED CUSTARD MADE OF WHOLE EGG LIQUID

Organoleptic characteristics*	Pasteurization techniques Conventional	Ultrapasteurization
Colour	8.66 ± 0.21	8.33 ± 0.21
Texture	7.83 ± 0.16	7.66 ± 0.21
Flavour	7.66 ± 0.21	7.50 ± 0.22
Overall acceptability	7.83 ± 0.16	7.66 ± 0.21

* 10-point scale was followed where 1 = Unacceptable, 10 = Excellent
Number of panelists = 6

However, the reduction in sponge cake volume observed in the present study was comparatively less in relation to earlier reports made by Cunningham (1977). The cake height was comparatively less under ultrapasteurization, when compared to conventional pasteurization (Table 2), agreeing with the observation already made by Martinez et al (1995).

Shape characteristics : The results presented in Table 3 indicated 2% and 0.6% sagging; 15% and 18% flatness and 83% and 81.33% roundness, respectively in Angel cakes and sponge cakes due to the effect of conventional pasteurization treatment (Table 3). On the other hand, the effect of ultrapasteurization showed 12.66% and 14.66%, sagging, 23.66% and 24% flatness and 63.66% and 61.33% roundness, respectively in case of Angel cakes and sponge cakes. These findings showed that ultrapasteurization had a significant (P<0.05) effect on shape characteristics of Angel cakes. Similar observations have been reported by Hanson et al (1947). The findings of the present study provided an indication that cake characteristics of sponge cakes made by ultrapasteurization method were inferior. Further work might be necessary to eliminate this by modifying the cake formulation to reduce the shape defects.

Coagulation properties of custards : There was a significantly (P<0.05) lower spread in ultrapasteurization, when compared to conventional pasteurization (Table 4), indicating more of coagulation with the rise in temperature in the ultrapasteurization. Coagulation increased as the temperature shifted from 65 to 70°C (Stadelman and Cotterill 1977) and 56 to 66°C (Baldwin 1977; Bergquist 1977).

No significant difference was observed between conventional and ultrapasteurization in the organoleptic scores of baked custards made of WEL (Table 5) in respect of colour, texture, flavour and overall acceptability. Earlier workers have also reported similar findings (Hanson et al. 1947; Miller and Winter, 1950; Ball et al. 1987).

The organoleptic qualities of sponge cakes made by WEL and yolk (Table 6) indicated significantly (P<0.05) lower scores for tenderness under ultrapsteurization treatment, when compared to conventional pasteurization. On the other hand, when yolk was used for sponge cakes under conventional and ultrapasteurization, there was no significant change in tenderness due to the pasteurization temperature. The texture scores of the sponge cakes made of both WEL and yolk were significantly (P<0.05) lower due to the effect of ultrapasteurization. These findings were consistent with those observed earlier by Cunningham (1977), Peleg and Normand (1983) and Hamid-Samimi et al (1984).

TABLE 6. EFFECT OF CONVENTIONAL VERSUS ULTRAPASTEURIZATION ON ORGANOLEPTIC QUALITY* OF SPONGE CAKES (MEAN ± SE)

Egg component	Treatment	Tenderness	Texture	Colour	Flavour
Whole egg liquid	Conventional pasteurization	8.00[b] ± 0.12	8.46[b] ± 0.10	8.33[a] ± 0.16	6.41[a] ± 0.15
	Ultrapasteurization	7.16[a] ± 0.10	7.61[a] ± 0.15	8.25[a] ± 0.17	7.41[b] ± 0.20
Yolk	Conventional pasteurization	7.25[a] ± 0.21	7.28[b] ± 0.08	7.41[a] ± 0.20	7.33[a] ± 0.16
	Ultrapasteurization	6.58[a] ± 0.23	6.83[a] ± 0.04	7.66[a] ± 0.16	7.41[a] ± 0.20

* 10-point scale was followed, where 1 = Unacceptable, 10 = Excellent

Figures with different superscripts in a column within egg component differ significantly (P<0.05)

Number of panelists = 6

TABLE 7. EFFECT OF CONVENTIONAL VERSUS ULTRAPASTEURIZATION ON MOISTURE ABSORPTION OF CAKES

Egg components	Pasteurization techniques	Types of cakes	Moisture, %
Whole egg liquid	Conventional pasteurization	Sponge cakes	22.28[b] ± 0.03
	Ultrapasteurization	"	15.28[a] ± 0.08
Liquid white	Conventional pasteurization	Angel cakes	30.43[b] ± 0.04
	Ultrapasteurization	"	20.32[a] ± 0.04
Yolk	Conventional pasteurization	Sponge cakes	57.46[b] ± 0.06
	Ultrapasteurization	"	44.82[a] ± 0.91

Figures with different superscripts in a column within egg component differ significantly (P<0.05)

Each value is a mean ± SE of three observations

Under ultrapasteurization condition, the colour score of sponge cakes from WEL was slightly (P<0.05) decreased to be in consistent with the earlier findings (Hanson et al. 1947; Miller and Winter (1950). Wong et al (1996) observed no difference in colour characteristics of pasteurized samples. Colour of sponge cakes made using yolk treated in ultrapasteurization process was slightly (P<0.05) improved (Table 6). Flavour characteristics of sponge cakes made up of WEL under ultrapasteurization improved significantly (P<0.05), while that made from yolk showed marginal (P<0.05) improvement. The observations were consistent with those of Miller and Winter (1950) and Ball et al (1987).

Sponge cakes and Angel cakes made by ultrapasteurization using WEL, yolk and LW had significantly (P<0.05) lower moisture absorption (Table 7) as compared to conventional pasteurization. This effect was further related to the changes in the cake volume and cake height in Angel cakes and sponge cakes (Tables 1 and 2), indicating that there was a direct relationship between lower moisture absorption and lower cake volume and height.

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RESEARCH PAPER

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Influence of Particle-Particle Interactions of Fluid-to-Particle Heat Transfer Coefficients (h_{fp}) Under Tube Flow Conditions Using Stationary Particle Technique

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Fluid-to-particle heat transfer coefficients (h_{fp}) were determined experimentally during flow of fluid over one or more particles using "stationary particle technique". The effects of different process parameters (e.g., particle-particle interaction, carrier fluid viscosity, carrier fluid temperature and flow rate) on h_{fp} were quantified. The h_{fp} value for the sample particle (silicone sphere) decreased by 5-20%, when another particle at different orientations (0°, 30°, 45° and 60°) was introduced upstream, whereas h_{fp} values increased by 14%, when multiple particles were introduced upstream of the sample particle (flow rate = 5.2 x 10^{-4} m^3/s and fluid temperature = 73.6°C). The h_{fp} value decreased three-folds with an increase in carrier fluid viscosity from 0.4 x 10^{-3} to 33.3 x 10^{-3} Pa•s. The h_{fp} values increased four- and two-folds with an increase in carrier fluid temperature (from 60° to 80°C) and flow rate (from 0.27 x 10^{-3} to 2.82 x 10^{-3} m^3/s), respectively.

Keywords : Particle-particle interaction, Sterilization, Aseptic processing, Stationary particle technique.

Aseptic processing is of great interest to the food industry because of its advantages over in-container sterilized, pasteurized-chilled, frozen or dehydrated foods. It ensures improved product quality, low energy consumption and reduction in waste generation. Design and optimization of aseptic processes for liquid foods with particles depend mainly on the value of fluid-to-particle heat transfer coefficient (h_{fp}). To calculate integrated lethality of a particle, its time-temperature history is used (Ball and Olson 1957):

$$F_o = \int_0^t 10^{\left(\frac{T-T_{ref}}{z}\right)} dt \qquad \text{- - - - - (1)}$$

where F_o = integrated lethality (s), t = total processing time (s), T = particle-temperature at time t (°C), T_{ref} = reference temperature (°C) and z = temperature change needed to change D-value (decimal reduction time, s) by 90% (°C).

Modelling of aseptic processing is necessary because monitoring of temperature history of particles is quite difficult, when the particles move in the aseptic processing systems. Modelling of the aseptic process schedules requires heating medium temperature and h_{fp} as input parameters so that time-temperature profiles for the particle can be predicted. This can be substituted in Eq. 1 to find the processing value (F_o). Extensive research has been done to determine the h_{fp} by using different techniques to monitor time-temperature data (Heppell 1985; Chandarana et al. 1990; Ramaswamy et al. 1992; Stofors and Merson 1990; Maesmans et al. 1992; Zitoun and Sastry 1994; Balasubramaniam and Sastry 1994 a, b, c, 1996 a, b Awuah et al. 1995). Recently, Palaniappan et al (1997 a, b) of Tetra Pak Research Centre, Buffalo Grove (IL) have filed a newly developed procedure for the microbiological validation of an aseptic particle processing system, with Food and Drug Administration (USA) and received approval for the first multiphase aseptic food process. It has been reported that commercial aseptic particle processing systems consist of multiple particles and presence of other particles may significantly affect the h_{fp} (Bhamidipati and Singh 1994). The objectives of the present work were : (i) to determine h_{fp} for a single particle in a multiple-particle system incorporating the effect of different orientations of particles on the h_{fp}, using "Stationary particle technique" and (ii) to determine the effects of process parameters such as carrier fluid viscosity, carrier fluid temperature and flow rate on the h_{fp} [It is acknowledged that in a real aseptic processing system, particles will flow freely along with the fluid, however, a conservative approach of "Stationary particle technique" was employed in the present study so as to obtain a "conservative" h_{fp} value. Such an approach is easy to adapt under a pilot scale scenario].

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Materials and Methods

Preparation of sample sphere : The silicone spheres (12.7 mm diameter) were fabricated using a specially designed mould, made of Teflon. The mould was split in two halves, allowing the placement of a thermocouple and a fishing wire through it. The spheres were made from a silicone rubber compound (General Electric, Waterford, NY), consisting of a base compound (RTV11) and a curing agent (Dibutyl tin dilaurate (DBT) (Table 1). The RTV 11 and DBT were mixed, as per the instructions given in the product data sheet (supplied by the manufacturer) in a mixing container 4-5 times larger than the volume of RTV11.

TABLE 1. PROPERTIES OF THE SILICONE BASED RUBBER COMPOUND (RTV11) WITH 0.5% DIBUTYL TIN DILAURATE (DBT) CURING AGENT (GENERAL ELECTRIC, WATERFORD, NY)

Property	Value
Specific gravity	1.19
Useful temperature range (°C)	–54 to 204
Thermal conductivity (k) ($W•m^{-1}•K^{-1}$)	0.2926
Specific heat (C_p) ($J•kg^{-1}•K^{-1}$)	1463

TABLE 2. MEASURING GUIDE FOR THE ADDITION OF THE CURING AGENT (DBT) TO SILICONE RUBBER BASE COMPOUND (RTV11) (GENERAL ELECTRIC, WATERFORD, NY)

RTV11 Mass, g	Number of drops for two DBT concentrations	
	0.1%	0.5%
100	5	25
454	23	115

Eight Grams of RTV11 was weighed and an appropriate amount (two drops) of curing agent (0.5% DBT by mass) was added to it (Table 2). The RTV11 and DBT were thoroughly mixed for about 5 min using a clean glass rod by scraping the sides and bottom of the container carefully to produce a homogeneous mixture. After mixing, a syringe was filled with the prepared mixture and was injected into the mould through a hole at the top of the mould. After 24 h, silicone sphere was removed out of the mould and was considered cured.

Preparation of carrier fluid : Water or carboxymethylcellulose (CMC) (Cabiochem Corporation, La Jolla, CA) solutions at different concentrations (0.2 and 0.4%) were used as carrier fluid. The CMC solutions were prepared in a tank equipped with a stirrer and a steam jacket. Measured amounts of CMC (400 and 800 g) were added to 200 L water at 65°C and the solution was stirred for 30 min (until CMC was mixed thoroughly as determined by visual inspection). The CMC solution was prepared for each experiment, i.e., it was not stored to be used for another experiment. The viscosities of the CMC solutions (0.2% and 0.4% at 73.6°C) was measured by Cannon-Fenske Routine viscometer according to the instructions supplied by the manufacturer (Cannon Instrument Co., State College, PA).

Experimental setup : Schematic of the experimental setup consisted of a storage tank, centrifugal pump, heat exchanger, temperature-controller, diversion valve and processing tube (Fig. 1). The centrifugal pump (Model N6124 FK11, Leeson Electric Co., Toronto, Ontario, Canada) pumped water from the storage tank to the plate type heat exchanger (M/C 5383, Type HX, 696-895, APV, London, England), which heated water and forwarded it to the processing tube. The temperature- controller (Anderson Instrument Co. Inc., Fultonville, NY) was used to set the temperature of the carrier fluid at a fixed temperature. The diversion valve (Model 1-1/2" FD-7500-5-240 w/6, Alloy Products Corp., Waukesha, WI) allowed the forward flow of the carrier fluid to the processing tube, when the temperature of the carrier fluid reached the set temperature.

The processing tube (46 mm inner diameter and 900 mm in length) was fabricated using stainless steel. Two posts were made inside the tube at both ends to attach the following wire, with sample sphere on it. Silicone spheres (12.7 mm diameter) were used in the experiments to simulate food particles. Spheres at different orientations (making angles of 0°, 30°, 45°, and 60° from the horizontal plane of the sample sphere) were also fabricated by attaching spheres on 2-mm-diameter aluminum stiff wires. Fig. 2 shows the sample particle along with the multiple particles in the processing tube. Particle (sphere) - centre temperature and fluid temperature were measured using Teflon-coated copper constantan thermocouple probes (Thermoelectric, Bradford, Ontario, Canada). Thermocouples were calibrated using calibration thermometers having a resolution of 0.1°C. It should, however, be noted that the thermocouples have an inherent error of about ± 0.5°C. A data acquisition system (Omega, 5508TC, Stanford, CT) was used to record time-temperature data at the centre of the sphere and the fluid temperature.

Experimental plan : The time-temperature data at the centre of the silicone spheres were acquired, when water of CMC solution was used as the carrier fluid for determination of h_{fp}. The experimental plan is summarised in Table 3.

Experimental procedure : Stationary particle technique (Sastry 1990; Balasubramaniam and Sastry 1994 c), which involves placement of a particle with an implanted temperature transducer in a flowing fluid stream and measurement of particle and fluid temperature during the experiment, was used to collect the time-temperature data at the centre of the particle. The thermocouple-embedded silicone-sphere was placed at the centre of the processing tube using a fishing wire, which was tied to the two end posts. The processing tube was connected to the experimental setup and the thermocouple was attached to the data acquisition system. The temperature of the carrier fluid was set to the desired level. Once the carrier fluid reached the set-temperature, it was diverted to the processing tube and the temperatures, at the centre of the sphere with time were recorded onto a disk using the data acquisition system. After

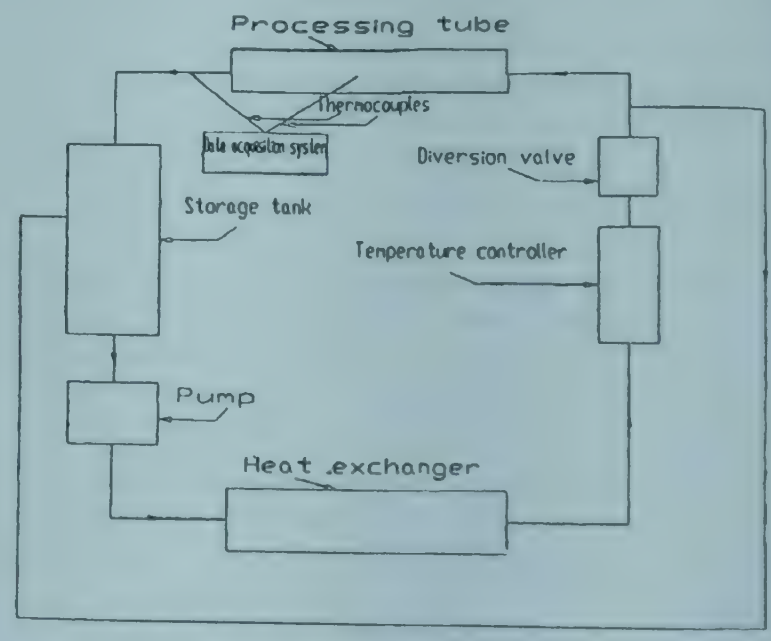


Fig. 1. Schematic diagram of the experimental setup.

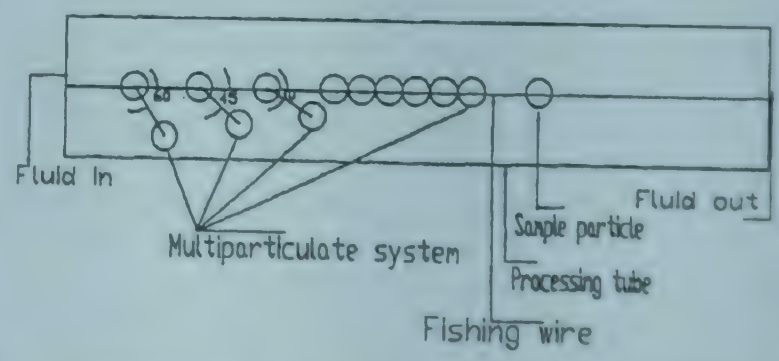


Fig. 2. Multiple-particle system (12 particles with the sample sphere) consisting of particles at different orientations (0°, 30°, 45° and 60°) in the processing tube

TABLE 3. EXPERIMENTAL PLAN FOR DETERMINATION OF h_{fp} AS IT IS AFFECTED BY PARTICLE-PARTICLE INTERACTION, CARRIER FLUID VISCOSITY, CARRIER FLUID TEMPERATURE, AND FLOW RATE

Parameter	Number of levels	Details of level
Sample sphere	1*ζ	Silicone-sphere with a thermocouple embedded at its centre (36 952)**
Sample sphere with spheres at different orientations	5*ζ	0°, 30°, 45°, 60° and multiple particles
CMC concentrations	3*ψ	0.0, 0.2, and 0.4%
Temperature of carrier fluid (flow rate = 2.82×10^{-3} m³/s)	3ψζ	60°, 70° and 85°C
Flow rate of carrier fluid (temperature = 70°C)	3ψζ	0.27×10^{-3}, 1.24×10^{-3}, and 2.82×10^{-3} m³/s

* Temperature was 73.6°C and flow rate of fluid was 5.2×10^{-4} m³/s

ζ Water was used as carrier fluid

ψ Only multiple particles were used for these tests

** Reynolds number based on pipe diameter and velocity of flow in the pipe

each experiment, the processing tube was cooled to room temperature using tap water and another sample particle was attached.

Mathematical treatment : The heat transfer to a sphere when dipped into a constant temperature medium is

$$\frac{\partial\theta}{\partial t} = \alpha\left(\frac{\partial^2\theta}{\partial r^2} + \frac{2}{r}\frac{\partial\theta}{\partial r}\right) \qquad \text{- - - - - (2)}$$

governed by (Incropera and Dewitt 1985) :

where $\theta = (T_\infty - T_r)/(T_\infty - T_i)$, t = time (s), r = distance from the centre of the sphere (m), T_∞ = temperature of the heating medium (°C), T_r = temperature of the sphere at time t and at a distance r (°C), T_i = initial temperature of the sphere (°C) and α = thermal diffusivity ($k/(\rho\ C_p)$) (m²/s).

For uniform initial temperature and convective boundary conditions, the analytical solution to Eq 2 is (Incropera and Dewitt 1985; Schneider 1955) :

$$\theta = \sum_{n=1}^{\infty} C_n \exp\ (-\xi_n^2 Fo)\ \frac{1}{\xi_n r^*} \sin(\xi_n r^*) \qquad \text{- - - - - (3)}$$

where Fo = Fourier number = $(\alpha\ t)/r_0^2$, r_0 = radius of sphere (m), $r^* = r/r_0$,

$$C_n = \frac{4[\sin(\xi_n) - \xi_n \cos(\xi_n)]}{2\xi_n - \sin(2\xi_n)} \qquad \text{- - - - - (4)}$$

and the discrete values of ξ_n are positive roots of the transcendental equation :

$$1 - \xi_n \cot(\xi_n) = Bi \qquad \text{- - - - - (5)}$$

where Bi = Biot number = $(h_{fp}\ r_0)/k$.

For a sphere, Heisler (1947) has shown that for Fo ≥ 0.2, the foregoing series solution (Eq 3) can be approximated by a single term :

$$\theta = \theta_0 \frac{1}{\xi_1 r^*} \sin(\xi_1 r^*) \qquad \text{- - - - - (6)}$$

where θ_0 = the temperature-ratio $((T_\infty - T_0)/(T_\infty - T_i))$ at the centre of the sphere (T_0 = temperature at the centre of a sphere at time t) and is of the form :

$$\theta_0 = C_1 \exp\ (-\xi_1^2 Fo) \qquad \text{- - - - - (7)}$$

Calculation of h_{fp} : Knowing the thermal diffusivity (α), radius of sphere (r_0), and time-temperature data at the centre of the sphere, C_1 and ξ_1 were obtained by performing non-linear regressions using SAS (1985) for each of the five replicates at different experimental conditions. The value of ξ_1 was substituted in Eq 5 to give Bi. From Bi, k, and r_0; h_{fp} was calculated. The reported h_{fp} is the mean of the five replicates at different experimental conditions. Also, C_1 and ξ_1 were used to back calculate predicted temperatures.

Results and Discussion

Effect of process parameters on the h_{fp} : The effect of process parameters such as particle-particle interaction, carrier fluid viscosity, carrier fluid temperature and flow rate on the h_{fp} were quantified. As shown below, the data fall partially in the range of heat transfer coefficients reported by earlier researchers [Chandarana et al. 1990: 66-107 W•m⁻²•K⁻¹; Mwangi et al. 1993: 59-1301 W•m⁻²•K⁻¹; Bhamidipati and Singh 1994: 108-196 W•m⁻²•K⁻¹;]. It is to be noted that experimental conditions and techniques used in different studies varied widely.

Particle-particle interaction : Convection is the main mode of heat transfer during aseptic processing of liquid foods with particles. The essential feature of a convective heat-transfer process is the transport of energy to or from a surface by both molecular conduction and gross fluid movement (Kays and Crawford 1993). Any change in the flow field around the particle results in thickening or thinning of the boundary layer around the particle, thereby decreasing or increasing the fluid-to-particle heat transfer.

The present study examined the effect of presence of particles at different orientations on the h_{fp} and thus indirectly, on the flow field around the sample particle. The h_{fp} values were significantly influenced by the presence of particles in the processing tube (Table 4). Table 5 gives Analysis of Variance (AVOVA) for the effect of particle-particle interaction on h_{fp}, using SAS (1985).

The h_{fp} values decreased slightly, when another particle at different orientations (0°, 30°, and 60°) was introduced upstream at a distance of 25.4 mm from the sample particle (it should be noted that an orientation of 45° gives an increase in h_{fp} value) (Table 4). A trend was expected because in the presence of another particle at different orientations, fluid velocity (v) is divided into two components (v cosβ and v sinβ, when β = angle of orientation of the particle). Therefore, the fluid velocity (v) approaching the sample particle decreases, which may have resulted in thickening of the boundary layer around the sample particle, thereby decreasing the h_{fp}. These results would help in making judgement of assigning h_{fp} value, while modelling aseptic process schedules in situations, where the

TABLE 4. FLUID-TO-PARTICLE HEAT TRANSFER COEFFICIENT (h_{fp}) FOR THE SAMPLE SPHERE ON ITS OWN AND IN THE PRESENCE OF OTHER PARTICLES AT DIFFERENT ORIENTATIONS (FLUID TEMPERATURE = 73.6°C AND FLOW RATE = 5.2 X 10^{-4} m^3/s)

	h_{fp}* ($W \cdot m^{-2} \cdot K^{-1}$)				
Sample sphere on its own	Sample sphere in presence of another particle at an orientation of				Sample sphere in a multiple particle system
	0°	30°	45°	60°	
154	130	122	146	125	176

*Mean value of five replicates

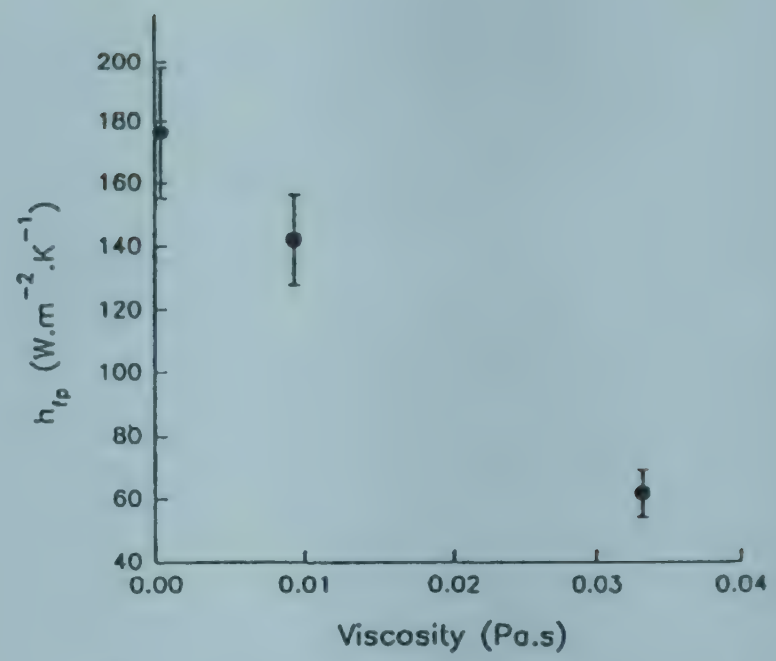
TABLE 5. ANALYSIS OF VARIANCE (ANOVA) FOR THE INDIVIDUAL EFFECTS OF PARTICLE-PARTICLE INTERACTION, FLUID VISCOSITY, FLUID TEMPERATURE AND FLOW RATE ON h_{fp} (T_3 SUMMARISES THE EXPERIMENTAL CONDITIONS FOR EACH PARAMETER).

Parameter	Degrees of freedom (DF)	ANOVA SS (Sum of squares)	Mean square	F Value	Pr>F (level of significance)
Particle-particle interaction	5	10990.29	2198.06	9.10	0.0001
Fluid-viscosity	2	39293.33	19646.67	9823.33	0.0001
Fluid-temperature	2	61423.33	30711.67	4606.75	0.0001
Fluid-flow	2	4343.33	2171.67	814.37	0.0001

sample particle is approached by other particles making similar angles from the horizontal plane of the sample particle. However, the experiments performed in the present study did not show my trend.

Analysis of variance (Table 5) shows that effect of particle-particle interaction on h_{fp} is highly significant (level of significance = 0.0001). Depending upon the flow rate, Mwangi et al (1993) also reported an enhancement in heat transfer between 80 and 200% with an increase in solid fraction (0-3.2%). This effect is caused by the flow field around a particle by the presence of other particles in the suspension at high Reynolds number. In the multiple-particle system, different velocity components (due to different orientations of particles) cause agitation or turbulence in the fluid flow. This agitation might have resulted in the thinning of the boundary layer around the sample particle, thereby increasing the h_{fp}. In addition, this effect is analogous with fluidization of particles, where the slip velocity increases with increasing solid fraction requiring less flow to suspend the particles.

Carrier fluid viscosity, carrier fluid temperature and flow rate : Fig. 3 shows that with an increase in the viscosity of the carrier fluid (CMC) from 0.4 X 10^{-3} Pa•s (0% CMC) to 33.33 X 10^{-3} Pa•s (0.4% CMC), h_{fp} values decreased from176 to 54 $W \cdot m^{-2} \cdot K^{-1}$ for the multiple-particle system (fluid-temperature = 73.6 ± 0.5°C, flow rate = 5.2 X 10^{-4} m^3/s). Equivalent change in Reynolds number (Re) was 36952 to 422. The higher fluid viscosity provides more resistance to fluid flow and thus decreases the agitation at the interface (thickening of the boundary layer around the particle), thereby decreasing the h_{fp}.



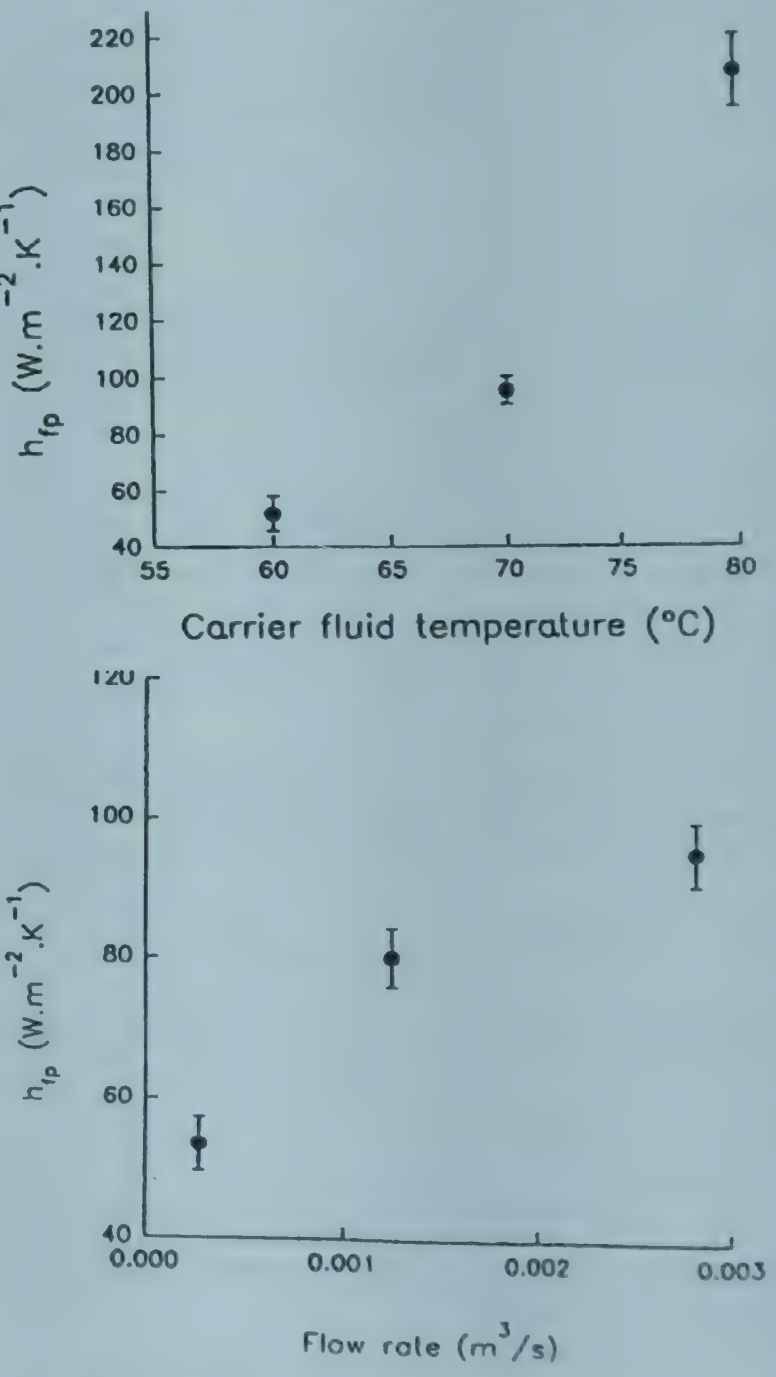


Fig. 3. Fluid-to-particle heat transfer coefficients (h_{fp}) ($W \cdot m^{-2} \cdot K^{-1}$), in the multiple-particle system at different viscosities (0.4 x 10^{-3}, 9.3 x 10^{-3} and 33.3 x 10^{-3} Pa•s), carrier fluid temperatures (60°, 70° and 80°C) and flow rates (0.27 x 10^{-3}, 1.24 x 10^{-3} and 2.82 x 10^{-3} m^3/s) (Table 2 summarises the experimental conditions for each experiment)

Fig 3 also shows that with an increase in carrier fluid (water) temperature from 60 to 80°C, h_{fp} values increased from 49 to 202 $W \cdot m^{-2} \cdot K^{-1}$ for the multiple-particle system at flow rate = 2.82 x $10^{-3} m^3/s$. By incorporating the effect of temperature on ρ (density) and μ (fluid viscosity), the Reynolds number (Re) based on pipe diameter changed from 163 285 to 216 724. At higher temperatures and more agitation, molecules of the carrier fluid have higher kinetic energy, thereby thinning the boundary layer at the particle interface and increasing the h_{fp}. In addition, multiple-particle-system resulted in fluidization of particles, which might have further enhanced the heat transfer phenomenon in case of forced convection. Fig 3 further shows that with an increase in flow rate from 0.27 x 10^{-3} (Re=18 068) to 2.72 x 10^{-3} (Re=188 710) m^3/s, h_{fp} values increased from 53 to 94 $W \cdot m^{-2} \cdot K^{-1}$ for the multiple-particle system (carrier fluid temperature = 70°C). The similar trends were also observed by earlier researchers (Zitoun and Sastry 1994; Balasubramaniam and Sastry 1994 a; Mwangi et al. 1993; Chang and Toledo 1989; Zuritz et al. 1990). Table 5 shows that the individual effects of fluid viscosity, fluid temperature, and flow rate on h_{fp} are highly significant (level of significance = 0.0001).

Conclusion

Effect of particle-particle interaction on h_{fp} was found to be highly significant (level of significance = 0.0001) during aseptic processing of liquid foods with particulate using "Stationary particle technique". It was also found that the process parameters (fluid viscosity, fluid temperature, and flow rate) affected the h_{fp} value significantly (level of significance = 0.0001). The h_{fp} value for the sample particle increased, when multiple-particles were introduced in the processing tube. Also, the h_{fp} value decreased with an increase in fluid viscosity; and increased with an increase in fluid temperature and flow rate.

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RESEARCH NOTE

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Standardization of Recipe for the Development of Plum Appetizer

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Efforts were made to develop nutritious and palatable plum appetizer. Two different combinations of fruit pulp and spice recipe were tried at three levels (40, 45 and 50°Brix) of total soluble solids for the standardization of the product. The prepared appetizers were evaluated for sensory attributes by a panel of semi-trained judges. A combination of 30% fruit pulp with standard recipe (0.1% cardamom, 0.25% cumin, 0.4% black pepper, 0.5% common salt, 1.0% black salt, 1.5% ginger extract and 1.0% mint extract) at 40°Brix TSS was found to be the best. The developed appetizer was also analyzed for various physico-chemical characteristics. The appetizer had appealing colour, body, flavour and 30.8 sugar/acid ratio. The sale price of appetizer was estimated to be Rs. 17.93 per 700 ml bottle.

Keywords : Plum, Appetizer, Sensory evaluation, Spices.

Plum (*Prunus salicina* L.) is one of the important temperate fruits grown in countries like USA, China, Germany and Romania. The world production of plums during 1997-98 was estimated to be 7,190,000 MT to which India contributed about 56,000 MT (FAO 1998). In India, it is predominantly grown in Jammu and Kashmir, Himachal Pradesh and hills of Uttar Pradesh and Tamil Nadu (Kishore et al. 1991).

Stone fruits constitute about 20% of the total fruit production in Himachal Pradesh, while plum accounts for 60% of the total stone fruit production (Anon 1988). Plums have attractive colour, flavour and taste and particularly rich in carbohydrates and minerals. The fruits are highly perishable and require immediate processing to avoid post-harvest losses. But, due to high acidity and astringency, their utilization in the preparation of products is limited. Much work has been done to develop plum juice and paste from 'Stanley' plums (Wang 1993; Siddiq et al. 1994). Joshi et al (1993) observed that not more than 40% plum juice can be used in sweetened plum beverage. Presently, spice-based beverages are gaining importance in the market place (Lal et al. 1999). However, addition of herbs extract to plum pulp might attract good response from the consumers as an appetizer. The literal meaning of appetizer is "which creates appetite for". Spices in foods are primarily used for their consistency of flavour, aroma and stability in storage and natural antioxidants, which have carminative properties and aid digestion through the stimulation of appetite (Griffin 1992). Keeping all these points in view, the present investigation was undertaken to standardize fruit pulp and total soluble solids with spices and herbs to develop palatable and acceptable plum appetizer.

Ripe plum fruits of cv. 'Santa Rosa' were procured from the Research Orchard of the University of Horticulture and Forestry, Nauni-Solan. Fruits were sorted and washed thoroughly with water to remove adherent foreign materials. Pulping was done using a pulper (B. Sen and Barry, 1/32 inch). Extracted pulp was filled in jerry cans of 20 litre capacity with 2000 ppm KMS, tightly sealed with cap and kept for further studies.

Spices and herbs : Two recipes were prepared by mixing different proportions of spices (Table 1). Cardamom, cumin and black pepper were dried in an oven at 60°C for 24 h. and ground in the Super Mixer Grinder (Model MX-1155). Black salt was broken into small pieces by hammer and ground in a pestle and mortar. Fresh ginger was washed thoroughly, peeled manually and passed through a screw type juice extractor to extract juice. Fresh mint leaves were washed, crushed in a blender and squeezed through muslin cloth to get extract. Experimental details of the products are given in Table 2.

Appetizers were prepared from fresh as well as preserved pulp. Preserved pulp was heated till whole of the SO_2 vapourized from the pulp. Sugar syrup of 60°Brix was prepared separately, strained through muslin cloth, cooled and added to the calculated quantity of pulp. Pre-determined quantities of spices as per recipes in Table 1 were boiled in 100 ml of water, strained through muslin cloth and added to the mixture of pulp and sugar

TABLE 1. PROPORTION OF SPICES AND HERBS USED FOR THE PREPARATION OF ONE LITRE PLUM APPETIZER

Particulars	Spice recipes	
	S_1	S_2 (Standard)
Cardamon, g	1.0	1.0
Cumin, g	2.5	2.5
Black pepper, g	2.5	4.0
Common salt, g	10.0	5.0
Black salt, g	5.0	10.0
Ginger extract, ml	10.0	15.0
Mint extract, ml	5.0	10.0

TABLE 2. EXPERIMENTAL DETAILS OF PLUM APPETIZERS

Treatments	Plum pulp, %	TSS, °Brix	Spice recipe, S_1/S_2
T_1	30	40	S_1
T_2	30	40	S_2
T_3	30	45	S_1
T_4	30	45	S_2
T_5	30	50	S_1
T_6	30	50	S_2
T_7	40	40	S_1
T_8	40	40	S_2
T_9	40	45	S_1
T_{10}	40	45	S_2
T_{11}	40	50	S_1
T_{12}	40	50	S_2

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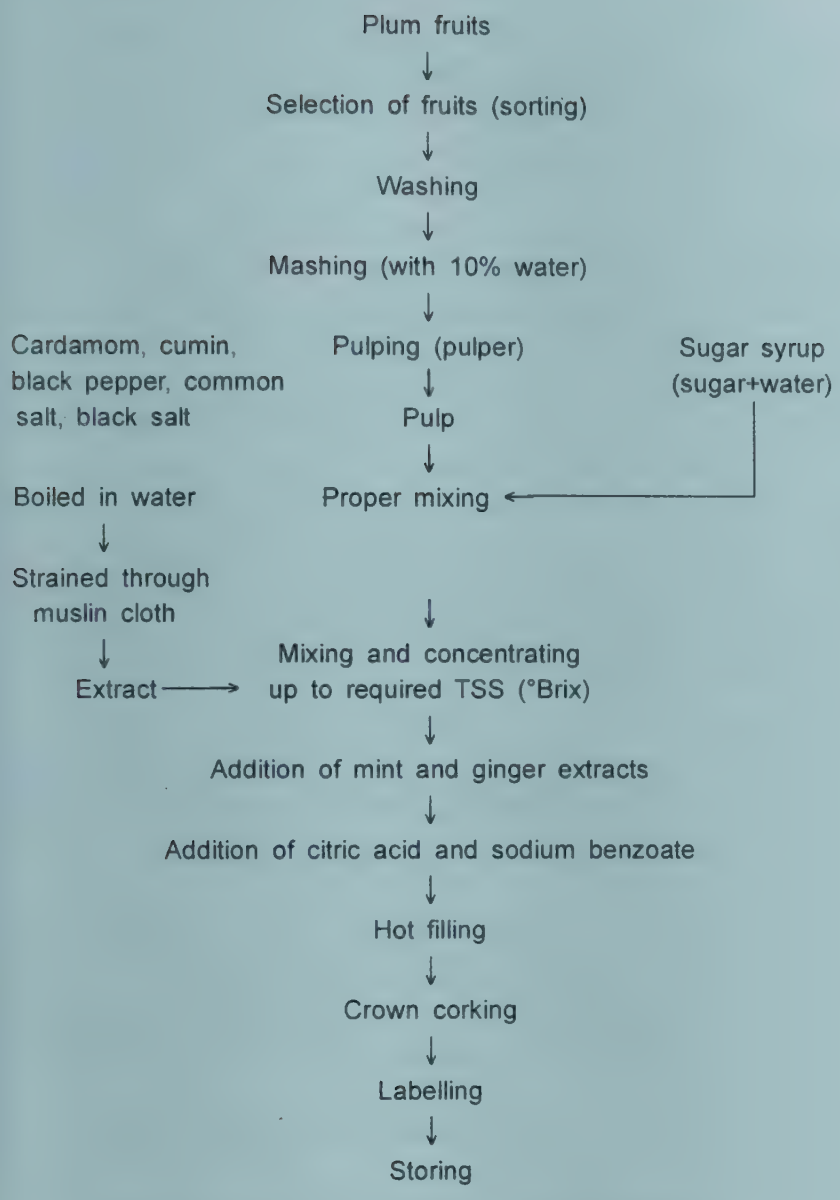

Fig. 1. Flow diagram for preparation of plum appetizer

syrup. Concentration of mixture was done upto the desired TSS as mentioned in Table 2 for different treatments. Finally, mint and ginger extracts were added as per recipes along with citric acid at 0.5% level and sodium benzoate at 0.06% level in the concentrated appetizer and mixed thoroughly. The appetizer was hot filled (85°C) in pre-cleaned sterilized glass bottles of 700 ml capacity, crown corked, labelled (Fig. 1) and kept for further analysis.

Chemical analysis : Total soluble solids (TSS) and pH were measured by a refractometer (Erma) and a pH meter (ELTOP-3030), respectively. Titratable acidity (TA), total solids, reducing sugars, total sugars (as invert sugars), ascorbic acid, salt (as NaCl) and tannins (as gallotannic acid) were estimated as per procedures described by Ranganna (1986). Energy value of the appetizer was calculated by taking into account the amount of sugar, protein and fat contents present in the appetizer. The contents of each nutrient were multiplied by a conversion factor as reported by Holland et al (1992).

Sensory evaluation : The sensory evaluation of the product was conducted by a panel of 25 semi-trained judges. Each sample was evaluated for colour, taste, flavour, body and overall acceptability on a 9-point Hedonic scale (Amerine et al. 1965).

Statistical analysis : The data were analyzed according to methods and procedures given by Panse and Sukhatme (1967).

Table 3 depicts the sensory evaluation scores for different quality parameters of plum appetizers.

Colour : The colour score (7.05) was highest for T_2 and lowest (6.50) for T_{11} (Table 3). T_2 was found statistically non-significant as compared to T_1, T_3, T_4, T_5 and T_6 and differed significantly with rest of the treatments. It was observed that with the increase in juice content and TSS (Table 2), colour score decreased significantly and this is in confirmity with the findings of Joshi et al (1993).

Flavour : The flavour scores for different treatments varied from 6.48 to 7.32 (Table 3). T_2 was non-significant as compared to T_1, T_3 and T_4 and differed significantly with rest of the treatments. Perusal of data revealed that the combined effect of spices and herbs by S_2 recipe improved the flavour in the final products. Among these, flavour of T_2 was adjudged the best due to balanced sugar/acid ratio of the blend, desirable aroma and taste (Chang et al. 1994). Lal et al (1999) also observed similar findings, while working with preparation and evaluation of apple and ginger based squash.

Taste : Maximum score (7.28) was recorded in T_2 and minimum score (6.50) was obtained by T_9 and T_{11} treatments. T_2 was found statistically non-significant, when compared to T_1 and T_3 and differed significantly with rest of the treatments. It can be concluded that products with high juice contents have high acid contents which might lower the acceptability for taste. Joshi et al (1993) also reported similar results.

Body : T_1, T_2, T_3, T_4, T_5, and T_6 treatments were statistically insignificant among one another and differed significantly with rest of the treatments. Maximum body score (7.00) was recorded with T_2 and minimum (6.58) was scored by T_{11}.

Overall acceptability : Highest score (7.32) was obtained by T_2 and lowest (6.50) was by T_{11}. Treatments T_1, T_3, T_4, T_5, T_6, T_7 and T_8 were found statistically non-significant with each other and differed significantly with rest of the treatments. T_2 treatment was adjudged the best by the panelists due to its acceptable acid/sugar blend. Addition of spices, herbs and salt might have influenced the taste perception of the judges (Lal et al. 1999; Joshi et al. 1993).

TABLE 3. SENSORY SCORES* OF DIFFERENT PLUM APPETIZERS

Treatments	Colour	Flavour	Taste	Body	Overall acceptability
T_1	6.95	7.05	7.05	6.95	6.98
T_2	7.05	7.32	7.28	7.00	7.32
T_3	6.88	7.02	7.00	6.75	6.90
T_4	6.75	7.10	6.95	6.80	6.95
T_5	6.80	6.72	6.65	6.76	6.80
T_6	6.90	6.76	6.68	6.78	6.85
T_7	6.67	6.70	6.65	6.70	6.70
T_8	6.72	6.73	6.70	6.71	6.72
T_9	6.65	6.55	6.50	6.62	6.58
T_{10}	6.68	6.75	6.65	6.65	6.62
T_{11}	6.50	6.48	6.50	6.58	6.50
T_{12}	6.55	6.58	6.56	6.60	6.52
$CD_{0.05}$	0.30	0.38	0.32	0.28	0.32

*On a 9-point Hedonic scale : 9 = Like extremely
1 = Dislike extremely

TABLE 4. PHYSICO-CHEMICAL CHARACTERISTICS OF PLUM FRUIT, PULP AND STANDARD APPETIZER

Characteristics	Fruit Mean ± SD	Pulp Mean ± SD	Appetizer Mean ± SD
TSS, °Brix	15.86 ± 0.06	15.08 ± 0.05	40.50 ± 0.05
Titratable acidity, % CA	2.08 ± 0.09	1.98 ± 0.04	1.05 ± 0.07
pH	3.02 ± 0.06	3.14 ± 0.05	2.58 ± 0.09
Total solids, %	16.34 ± 0.05	15.65 ± 0.04	46.62 ± 0.43
Reducing sugars, %	5.94 ± 0.20	5.28 ± 0.14	18.70 ± 0.24
Total sugars, %	7.98 ± 0.28	7.02 ± 0.22	32.34 ± 0.31
Sugar/acid ratio	3.83	3.54	30.80
Ascorbic acid, mg/100g	6.62 ± 0.07	5.80 ± 0.06	1.65 ± 0.08
Ash, %	0.32 ± 0.02	0.30 ± 0.02	0.14 ± 0.02
Anthocyanin, mg/100g	912 ± 4.10	858 ± 3.70	254 ± 3.35
Tannins, %	0.16 ± 0.08	0.12 ± 0.06	0.20 ± 0.03
Salt, %	–	–	0.90 ± 0.04
Energy value, Kcal/100g	–	–	129.71 ± 3.65

SD = Standard deviation

TABLE 5. COST OF PRODUCTION OF STANDARD PLUM APPETIZER

Ingredients	Rate Rs	Standard appetizer Quantity	Amount, Rs
Plum fruit	5/kg	4.0 kg	20.00
Sugar	18/kg	3.7 kg	66.60
PP caps	0.6/cap	14 Nos.	8.40
Cardamom	120/kg	10 g	1.20
Cumin	100/kg	25 g	2.50
Black pepper	270/kg	40 g	10.80
Common salt	6/kg	50 g	0.30
Black salt	8/kg	100 g	0.80
Ginger	20/kg	500 g	10.00
Mint	10/kg	60 g	6.00
Labels	0.35/lab.	14 Nos.	4.90
Citric acid	120/kg	50 g	6.00
Sodium benzoate	0.28/g	6.5 g	1.82
Bottles (700 ml)	2.5/bottle	14 Nos.	35.00
Total cost of ingredients			174.32
Processing cost	at 20%		34.86
Total preparation cost			209.18
Profit	at 20%		41.84
Net cost			251.02
Total yield	14 bottles		
Sale price / bottles			17.93

Physico-chemical characteristics of organoleptically best rated plum appetizer : Organoleptically rated best appetizer (T_2) was analyzed for various physico-chemical characteristics and the results are summarized in Table 4. TSS of standard plum appetizer was found to be 40.50°Brix, which was in confirmity with the FPO specifications. The titratable acidity of plum fruit and appetizer were 2.08 ± 0.09 and 1.05 ± 0.07%, respectively. Joshi et al (1993) evaluated enzymatically extracted plum juice for the preparation of beverages and reported similar findings with the prepared product. Total sugars of 7.98 ± 0.28 and 32.34 ± 0.31% and reducing sugars of 5.94 ± 0.20 and 18.70 ± 0.24% were obtained for plum fruit and appetizer, respectively (Table 4). Ascorbic acid content in developed plum appetizer was 1.65 ± 0.08 mg compared to 5.80 ± 0.06 mg in plum pulp. Anthocyanin contents of plum fruit, pulp and appetizer were 912 ± 4.10, 857.60 ± 3.70 and 254 ± 3.35 mg/100 g respectively. Tannin contents were 0.16 ± 0.08, 0.12 ± 0.06 and 0.20 ± 0.03% for plum fruit, pulp and appetizer, respectively. Addition of spices and herbs might have contributed to the phenolic contents of appetizer. Joshi et al (1991) and Attri et al (1994) also recorded an increase in phenolic content in the vermouth by addition of spices and herbs extracts. Calculated energy value of standard plum appetizer was 129.71 kcal/100 g.

Cost of production : The cost of production was calculated on the basis of current market prices of ingredients, nominal processing charges and reasonable profit margins (Table 5). The product was prepared on bulk (a lot of 10000 ml) and filled in standard beverage bottles of 700 ml capacity. The sale price was worked out to be Rs. 17.93 per 700 ml bottle.

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RESEARCH NOTE

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Firmness Studies on Bread Incorporated with Xanthan Gum

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Incorporation of xanthan gum at 0.1 to 0.5% levels has been investigated to evaluate its effect on the bread making and firmness properties of wheat 'WH 542' flour. It significantly improved the yield of bread. Firmness properties determined using Instron Universal Testing Machine revealed the bread to be significantly softer as a result of xanthan gum incorporation at each storage period. However stored wheat flour gave firmer breads.

Keywords : Wheat, Bread, Yield, Instron, Firmness.

Freshness is one of the primary factors favoured by the consumer to assess the quality of bread. Rheological properties of the crumb have been studied extensively, with major emphasis on the effect of rate of staling on bread firmness (Kamel et al. 1984). Bice and Geddes (1949) reported that firmness and crumbliness are closely related to organoleptic assessment of staleness. Bread firmness is influenced by a variety of factors, including product formulation (Marston and Short 1969; Short and Roberts 1971; Stutz et al. 1973; McDermott 1974; Tenny 1978). Increased skim milk solids, significantly increased the softness of the crumb (Platt and Powers 1940). Lorenz and Dilsaver (1982) showed that slice thickness and storage temperature affected compressibility. Short and Roberts (1971) and McDermott (1974) found that crumb firmness varied throughout the loaf, with the firmest felt at the centre of the loaf. Platt and Powers (1940) and Ponte et al (1962) reported that the centre slice of the loaf had a firmer crumb than slices near the end of a loaf and warned that the slices towards the end tended to be most irregular in compressibility.

Laboratory measurements of the softness of bread and cakes have been conducted by Baker's Compressimeter (Platt and Powers 1940; Noznick and Geddes 1943; Crossland and Favour 1950). Many workers have studied the effect of different UTM testing conditions (Bashfold and Hartung 1976; Bourne 1979; Kamel et al. 1984; Baker et al. 1986 a, b; Hibbard and Parker 1985; Redlinger et al. 1985; Lorenz and Dilsaver 1982). Based on their work, a standard method for measuring the crumb firmness of bread by universal Testing Machine has been accepted by American Association of Cereal Chemists in 1986. The present investigation was undertaken to determine the firmness studies on bread incorporated with xanthan gum.

Preparation of sample : Wheat samples 'WH 542' were procured from the 1996-97 harvest of Punjab Agricultural University, Ludhiana. Samples were conditioned to 14% moisture content for 24 h and milled using laboratory mill (Brabender Quadrumat Junior) to get 70% extraction wheat flour. Milled sampls were stored in pearl pet containers for further use. Xanthan gum was procured from John Baker Inc. Colorado USA.

Chemical properties : The chemical properties were determined using standard AACC (1990) procedures. All analyses were done in triplicate and their average values have been reported.

Break baking : Straight dough formulation was used in bread baking. Xanthan gum was dispersed uniformly in water for 60 min to accomplish hydration and added to the flour at the desired levels. Breads were prepared after adding 0.1, 0.2, 0.3, 0.4 and 0.5% xanthan gum on the basis of flour. The ingredients i.e., flour (200 g), water (variable), fresh compressed yeast (6 g), salt (2 g), sugar (10 g) and xanthan gum (variable) were mixed in a laboratory mixer (National Manufacturing Co. Nebraska, Lincoln) for 1.5 min and fermented at 30°C and 85% RH in the fermentation cabinet (National Manufacturing Co. Nebraska, Lincoln) for 90 min. After punching, second fermentation was carried out for 30 min. Then, the loaves were mechanically molded and proofed at 30°C and 85% RH for 25 min. Baking time was 25 min at 220°C in a rotary oven (National Manufacturing Co. Nebraska, Lincoln). Loaf volume was measured using rapeseed displacement method of Malloch and Cook (1930) one hour after baking. Evaluation of breads was carried out for crust and crumb characteristics i.e., texture, freshness score and eating quality by a panel of 6 semi-trained judges 24 h after the preparation (Irvine and McMullan 1960). The texture was evaluated in terms of softness and freshness on a scale of 1 to 6 with 6 for very fresh and 1 for stale and eating quality as normal or gummy. The unsliced loaves were placed in polyethylene bags, coded and stored at 27°C and 65% RH conditions till use.

Firmness measurement : After discarding the two outside portions (approximately 6 cm at each end), the loaves were sliced into 25 mm thick slices and used immediately for measuring firmness on Instron (Model 4464) Universal Testing Machine (Instron Corp., Canton, MA, USA). The rate of compression or crosshead speed was set at 100 mm/min. Flat aluminium plunger with a diameter of 36 mm (1017.9 mm^2), was used to compress the 25 mm thick slice. Slices were compressed to a total compression of 25% according to AACC (1990) procedure. The whole procedure was repeated on three different slices and the firmness values were averaged. The bread firmness measurements were also carried out at 24, 48, 72 and 96 h after baking. The softness index was calculated for 24, 48, 72 and 96 h sample by dividing the compression values at each time interval by the control (0 h).

Statistical analysis : A two way analysis of variance was

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TABLE 1 EFFECT OF XANTHAN GUM ON BREAD MAKING QUALITY

Xanthan gum, %	Loaf				Crumb		
	Weight, g	Volume, ml	Specific volume, ml/g	Extra yield, %	Texture	Score	Eating quality
–	302[a]	925[a]	3.06[a]	–	Soft	5	Normal
0.1	304[b]	980[b]	3.22[b]	0.6[a]	Soft	5	Normal
0.2	305[c]	1015[c]	3.33[c]	0.9[b]	Soft	5	Normal
0.3	306[d]	1025[d]	3.35[d]	1.3[c]	Very soft	5	Slightly gummy
0.4	307[e]	1040[e]	3.39[e]	1.6[d]	Very soft	5	Slightly gummy
0.5	308[f]	1085[f]	3.52[f]	2.0[e]	Very soft	5	Slightly gummy
MSD	0.87	1.96	0.05	0.02			

Means superscripted with different alphabets in columns differ significantly ($p<0.05$)

TABLE 2. MEAN FIRMNESS AND SOFTNESS INDEX VALUES FOR CRUMB SLICES FROM THE VARIOUS BREAD SAMPLES (n=3)

Xanthan gum, %	Firmness (g Force) Storage time, h						Softness Index Storage time, h			
	2	24	48	72	96	Mean	24	48	72	96
–	485	792	891	1036	1072	855[a]	1.63	1.84	2.14	2.21
0.1	437	727	901	1162	1228	891[b]	1.66	2.06	2.65	2.81
0.2	421	671	854	970	977	779[c]	1.59	2.02	2.65	2.32
0.3	404	637	850	958	954	761[c]	1.57	2.10	2.37	2.36
0.4	395	633	809	870	947	731[d]	1.60	2.04	2.20	2.39
0.5	335	602	755	835	902	686[e]	1.79	2.25	2.49	2.69
Mean	413[a]	677[b]	843[c]	972[d]	1013[e]					

Means superscripted with different alphabets in column and row differ significantly ($p<0.05$)

conducted on weight, volume and firmness data according to procedures described by Steel and Torrie (1960) to evaluate the effect of levels of xanthan gum as well as storage intervals for statistical significance. Least significant difference (LSD) was calculated for the significant effects.

Proximate composition of flour : Chemical characteristics of wheat flour indicated the wheat to be medium strong, containing 0.55% ash, 9.2% proteins (N X 5.7), 30.7% wet and 11.2% dry gluten, 35 ml SDS-Sedimentation value. The flour had low alpha amylase activity as revealed by Falling number (712), diastase activity (410 mg per 10 g flour) and damaged starch content (6.2%).

Organoleptic evaluation : The data for loaf and crumb quality characteristics of the various samples are shown in Table 1. The weight and volume of bread improved significantly ($p<0.05$) with the increase in level of xanthan gum from 0.1 to 0.5% as compared to control. This increase may be attributed to maximum water binding capacity of xanthan gum (232 ml/g) as compared to other gums (Sanchez et al. 1995). The crust shape was normal and the crust colour was golden brown for the breads, resulting from all the experimental parameters. The crumb was creamish white with medium fine uniform grain in all the cases. The texture of the breads improved from soft for 0.1 and 0.2% levels to very soft at 0.3-0.5% addition of xanthan gum. Rao Jyothsna et al (1993) reported that bread with normal eating quality, soft texture and yield could be increased by 3.5% upto 0.3% incorporation of xanthan gum.

Firmness evaluation : Mean firmness values (g), softness index for the various flour samples and analysis of variance data for the weight, volume and firmness are given in Tables 2 and 3, respectively. Analysis of variance revealed that the levels of xanthan gum had a significant effect on weight and volume, while the firmness was slightly ($p<0.05$) affected by both the levels of gum as well as storage time. A significant ($p<0.05$) effect of storage and levels indicated that xanthan gum had a pronounced effect on the firmness properties of bread Higher levels of gum incorporation performed better, giving improved bread quality with softer crumb as compared to control. Storage resulted in increased firmness of bread as

TABLE 3. ANALYSIS OF VARIANCE FOR WEIGHT, VOLUME AND FIRMNESS AS INFLUENCED BY LEVEL OF XANTHAN GUM AND STORAGE TIME.

Source	Degree of freedom	Mean square	LSD
		Weight	
Levels	5	14*	0.87
Error	12	0.7	
		Volume	
Levels	5	8930*	1.96
Error	20	1	
		Firmness	
Storage	4	360913*	33.31
Levels	5	35575*	29.80
Error	20	510	

*$p<0.05$

revealed by higher softness indices (Table 2). However, the storage effect on firmness could be reduced with increased levels of xanthan gum. The ability of xanthan gum to retain moisture and form complex with starch is of importance and inhibited retrogradation, consequently leading to extension of shelf life of baked goods (Pettitt 1982). As evident from Table 2, the firmness differed significantly ($p<0.05$) from control at all the stages of storage and time.

It may be concluded that the yield (specific volume) of the bread and softness of crumb can be improved by incorporating 0.2% level of xanthan gum in a medium strong wheat flour. The use of xanthan gum has been approved by FDA, as a stabiliser, emulsifier, thickener, suspender, bodying agent or foam enhancer in foods (Glicksman 1969).

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RESEARCH NOTE

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A Comparison of Different Virulence Markers of *Yersinia enterocolitica*

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Fifty eight *Yersinia* isolates of milk origin were evaluated for their virulence potential using different established virulence markers. On the basis of colonial morphology on trypticase-soy-agar, only six isolates producing rough margins could be designated as virulent. While congo-red uptake and calcium-dependent growth revealed four isolates as virulent, at least 19 isolates were identified as virulent on the basis of crystal violet binding. Auto-agglutination, at the same time, confirmed the virulence in the case of only seven isolates Thus, no correlation could be established amongst the different markers of virulence of *Y. enterocolitica*. Hence, none of the tests alone could be conclusively used for identifying virulent isolates.

Keywords : *Yersinia enterocolitica*, Virulence markers, Auto-agglutination.

Yersinia enterocolitica is an emerging pathogen of concern and is considered to be of major public health significance in dairy products. Pathogenic strains of the organism are etiological agents of a range of clinical entities in human, although acute non-complicated enteritis is by far the most frequent manifestation. On consumption of products that are contaminated with virulent strains of *Y. enterocolitica*, the organism enters the digestive tract and cause gastroenteritis. The illness appears to be frequent in children and adolescents but can also occur in adults. However, it is important to recognize that, adequate number of actively growing virulent bacteria is essential to cause the disease. The fact that the diverse foods are eaten regularly without any ill effects further demonstrates that, not all types of *Y. enterocolitica* are virulent and capable of causing illness. A number of bio-typing and sero-typing schemes have been suggested for establishing the virulence of *Y. enterocolitica* (Bercovier and Mollaret 1984; Cornelis et al. 1981; Kandolo and Wauters 1985; Wauters et al. 1987) in addition to several tests using invasiveness in cultured mammalian cells (Sai et al. 1975; Lee et al. 1977; Pederson 1979; Bernet et al. 1993).

Since these bio-typing schemes and techniques for evaluation of invasiveness of *Y. enterocolitica* strains using mammalian cells is highly complex, a number of alternate tests have been recommended for testing the virulence of these isolates. These include colony morphology (Scheimann 1989), calcium-dependent growth (Gemski et al. 1980), crystal violet binding (Bahaduri et al. 1987), congo-red binding (Prpic et al. 1983) and auto-agglutination (Laird and Cavanaugh 1980). However, a lot of variation has been reported in these tests, which are treated as markers of virulence. Since, not much information is available on the comparative utility of these virulence markers as reliable tests of pathogenicity, the present investigation was undertaken to evaluate the virulence of *Y. enterocolitica* strains isolated from milk.

A total of 110 milk samples were screened for *Y. enterocolitica* by selective plating on Cefsulodin-Irgasan-Novobiocin (CIN) agar (Kushal 1998). These strains were biochemically characterized using standard identification schemes (Marshall 1992). A total of 58 isolates thus identified as typical *Yersinia*, were evaluated for virulence using different markers of virulence, as described below.

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For colony morphology on Trypticase-soy-agar (TSA), the 24 h - old Brain-heart-infusion (BHI) broth cultures of the isolates were streaked on TSA and the plates were examined for irregular colonies indicating virulence (Scheimann 1989). To screen for crystal violet binding, the growth on TSA was flooded with one per cent crystal violet solution and examined for binding of the dye to the colonies (Bahaduri et al. 1987). Similarly, for congo-red uptake, the isolates were cultured on calcium-deficient agarose medium containing congo-red dye. After incubation for 24 h at 25°C, the plates were examined for tiny red colonies of pathogenic strains, which could be easily distinguished from the larger white colonies of non-pathogenic strains (Prpic et al. 1983). The minute size of the colonies was also indicative of the calcium dependency of the pathogenic strains of *Y. enterocolitica*. For the auto-agglutination test, the 24 h - old BHI culture isolates were inoculated into Methyl Red-Voges Proskauer (MR-VP) broth and incubated at 35°C for 24 h. The tubes were observed for agglutination (clumping) of bacteria along the wall and/or bottom with clear supernatant fluid. Isolates giving positive results were considered to be presumptively positive for virulence (Marshall 1992).

The present findings with regard to the colony morphology on Trypticase-soy-agar revealed only six isolates that produced colonies with irregular margins, six isolates forming both smooth and rough colonies and rest of the 46 isolates produced smooth colonies (Table 1). The rough and irregular margins were considered as indirect markers of virulence (Scheimann 1989).

Results revealed that, in the case of congo-red uptake, only 4 isolates were found to be positive. The small pink colonies of these positive isolates were also indicative of calcium dependent growth. These two factors have earlier been shown to be associated with the virulence potential of *Y. enterocolitica* (Varnam and Evans 1991). The role of calcium in providing stimulus during regulation was reported to be a distinct feature of *Yersinia*, that also differentiated them from other enteric pathogens (Cornelis et al. 1981). However, variation has previously been reported for congo-red uptake (Prpic et al. 1983). Similarly, crystal violet uptake has also been reported to be associated with virulence (Bahaduri et al. 1987). In the present study, only 19 of the 58 isolates indicated crystal violet binding.

TABLE 1. SCREENING OF *YERSINIA* ISOLATES WITH MARKERS OF VIRULENCE

Colony morphology	Crystal violet uptake	Congo red uptake	Calcium dependent growth	Auto-agglutination
S	–	–	–	–
S	–	–	–	–
S	–	–	–	–
S	–	–	–	–
S	–	–	–	–
S	–	–	–	–
S	–	–	–	–
S	–	–	–	–
S	–	–	–	–
S	+	–	–	–
S	–	–	–	–
S	+	–	–	–
S	+	–	–	–
S	–	+	+	–
S	+	–	–	–
S	+	–	–	–
S	–	–	–	–
S	–	–	–	–
S/R	–	–	–	–
S	–	–	–	–
S	+	–	–	–
S	+	–	–	–
S	–	–	–	+
R	–	–	–	+
S	–	–	–	–
S	–	–	–	–
R	+	–	–	–
R	+	–	–	–
S/R	–	–	–	–
S	–	–	–	–
S	–	–	–	–
S	–	–	–	–
S	–	–	–	+
R	–	–	–	–
S	–	–	–	–
S	–	–	–	–
S	–	–	–	–
S	–	–	–	+
R	–	+	+	+
S	+	–	–	–
S	–	–	–	–
S	–	–	–	–
S	–	–	–	–
S	+	–	–	–
S	–	–	–	–
S/R	+	–	–	–
S	–	–	–	–
S	–	–	–	–

Table 1 Cont'd

Colony morphology	Crystal violet uptake	Congo red uptake	Calcium dependent growth	Auto-agglutination
S	+	–	–	–
S/R	–	–	–	+
S/R	–	–	–	–
S	+	–	–	–
S	+	+	+	–
S	+	+	+	–
S/R	+	–	–	+
S	+	–	–	–

S = Smooth, entire margin

R = Rough, irregular margin

Some previous studies have also shown auto-agglutination as a positive indication of the virulence (Marshall 1992). In the present investigation, only seven strains were positive for auto-agglutination test at 36°C (Table 1). However, the clumping factors have been reported to be highly variable (Warnken et al. 1987) and even some biochemical atypical non-pathogenic strains were also found to show agglutination (Scheimann 1989).

Thus, on the basis of the present studies, no correlation could be established amongst different markers of virulence. Hence, these tests alone could not be conclusively used for establishing virulence of *Y. enterocolitica* isolates.

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RESEARCH NOTE

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Development of Cold Grinding Process, Packaging and Storage of Cumin Powder

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In this study, cumin was subjected to conventional grinding during which the mill and product temperatures rose to as high as 95°C and at this temperature, considerable loss in volatile oil and changes in the volatile constituents occurred. Lowering the temperature of the grinding zone by circulating chilled water (10-25°C) had profound influence on the retention of volatile oil, fineness of particles and sensory qualities of the ground cumin. Aluminium / low density laminated pouches and a storage condition of 37°C/ 70% RH could be used for packaging and storage of cold ground cumin.

Keywords : Cold grinding, Cumin, Heat transfer co-efficient, Packaging and storage.

Spices are valued for their flavour with both aromatic volatile and non-volatile constituents, which impart the desired flavour. Grinding of spices is an important step in processing during which losses of volatile oil and aroma occur. The technology used at present on grinding of spices has inherent disadvantages of high heat generation, loss of volatile oil and low efficiency. Therefore, this method of grinding is not desirable for materials of plant origin including spices with high heat sensitivity, high fat and fibre contents (Wistreich and Sachfer 1962). Loss of volatile oil could be overcome partly by lowering or maintaining the mill temperature as low as possible (less than the boiling temperature of the volatile constituents of the spices volatile oil). Grinding the spices in the cryogenic condition and to some extent by circulating the chilled water around the grinding zone are some of the methods suggested (Murthy et al. 1996). The cryogenic technology has some limitations related to its operational costs as well as capital investment and easier operating system.

The present investigation was undertaken to make a systematic study on cold grinding process, packaging and storage of ground cumin.

Freshly harvested dry cumin seeds (*Cuminum cyminum* L) were procured from the local market. The seeds were cleaned and stored in the air tight aluminium containers and used as and when required for the experiment.

An electrical grinder (Waring blender type) was used for grinding the seeds. The stainless steel jar of the grinder was modified by providing an insulated outer jacket, which held the cold water. For cold grinding, chilled water was circulated around the jar using a centrifugal pump (0.5 hp). The flow rate of the chilled water was adjusted using a needle valve. The temperatures of the water (10, 15 and 25°C) were adjusted by mixing the ice cold water and water at ambient temperature and the data were analysed statistically on a completely randomized design using analysis of variance technique (Snedecor and Cochran 1968) to find, if the differences were significant or not at 5% level significance.

Prediction of shelf life of ground cumin in different packaging materials : Both CON-S and CG-S samples were packed in low density polyethylene/aluminium foil (0.8μ) laminated pouches and stored in the desiccators at 25°C/90% RH and 37°C and 70% RH. The relative humidities in the desiccators were maintained by sulphuric acid solutions (Ruegg 1980). The shelf life of ground cumin packed in various packaging materials was predicted using an equation - In Me–Mc / Me – Mo = t. where, Me, Mc, Mo and t = the equilibrium moisture content, critical moisture content, initial moisture content and shelf life in weeks, respectively. Critical moisture content was calculated based on critical relative humidity using Henderson's equation (Henderson 1952).

The volatile oil contents and fineness modulus data of cumin, are given in Table 1. The volatile oil content of CG-S increased (28%) in comparison to the sample ground at 35°C (control). This was expected because grinding at higher temperature would result in the loss of volatile oil (Pruthi 1980; Murthy et al. 1996). The fineness modulus value of cold ground samples was decreased from 3.51 to 2.91, when temperature of the grinding zone was lowered from 35°C to 10°C. The lower fineness value of the powder would indicate fine particle size. The grinding operation conducted at 35°C increased the product temperature (67°C) and there was frequent stopping of the grinding blades because of "gumming up". In this temperature, the size reduction was more of tearing than shattering, which led to coarser ground products (Anon 1962).

Spices are valued for their aroma and visual quality. Visual examination of the ground products indicated that CON-S samples were slightly darker in colour compared to CG-S samples. Cake formation of CON-S samples was also observed during grinding.

TABLE 1. EFFECT OF GRINDING TEMPERATURES ON THE % VOLATILE OIL CONTENTS AND FINENESS MODULUS OF GROUND CUMIN

Temp of water, °C	Product temp, °C	Volatile oil content %	Fineness modulus
10	20.40	3.0	2.91
15	23.10	3.0	3.01
25	25.00	2.5	3.30
35	69.00	2.5	3.51

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Sensory scores for aroma of emulsion prepared based on volatile oil of both CON-S and CG-S samples indicated that sensory qualities of CG-S samples were better in comparison to the CON-S samples (Table 2). Statistical analysis indicated that the aroma of emulsion differed significantly ($P<0.05$). This could be inferred that cold ground spice samples contain more volatile compounds than those of CON-S samples. Pesck et al (1985) and Murthy et al (1996) observed better retention of volatile constituents of volatile oil of cold and cryo-ground spice samples of pepper, cumin and orgegano.

The initial moisture contents, total plate counts and free fatty acid contents of both CON-S and CG-S were 9.5% (d.b), 80 x 10^4 cfu/g and 0.11%, respectively. Changes in moisture, free fatty acid contents and total plate counts of CON-S and CG-S samples packed in various packaging materials and stored at 25°C / 90% RH and 37°C / 70% RH for four months are shown in Table 3. The increases in the moisture contents of the samples packed in the Al / ploy laminate pouches were less in comparison to the samples packed in the low density polyethylene and cellophane. CG-S and CON-S packed in Al / poly laminate had 21.26% and 22.10% moisture increases at 25°C / 90% RH, while it had 17.05 and 20.0% ambient temperature. The temperature of inlet temperature, outlet water and surface of the stainless steel jar were monitored, using metallic probes (1.5 mm dia) connected with multi-channel digital temperature indicator (Model 44/E Century Instrument).

Twenty five grams of dried cumin seeds were taken into the stainless jar of the grinder and conditioned with the temperature of the circulating water. Grinding speed of 1200 rpm and a period of 3 min. were fixed for each set of experiments. The ground cumin samples were quickly collected in the aluminium boxes and used for further experiment. In another set of experiment (control), samples were ground in the grinder (without circulating cold water) at room temperature (35°C). Moisture and volatile oil contents of both cold ground samples (CG-S) and control samples (CON-S) were determined by following ASTA (1968) methods.

Fineness of the ground product was determined by standard method of sieve analysis and fineness modulus was calculated (Pandey 1994).

Total plate counts of the ground sample were enumerated by standard method (Ranganna 1979). Fatty acid contents of the samples were determined by following AOAC (1984) method. Sensory evaluation of volatile oil of both CON-S and CG-S samples were done by following the method of Pesck et al (1985) with some modifications. In this, an emulsion of volatile oil with salt solution (0.1%) and corn flour (1.0 g) was prepared and served hot to 10 panelists. The panelists were requested to record their rating for overall acceptability on a 9 - point Hedonic scale using numerical values ranging from 1 to 9, where 1 represented 'dislike extremely' and 9 represented 'like extremely'. The data increase at 37°C / 70% RH. The storage stability or shelf life of packed ground cumin was influenced by the increase in moisture content during storage.

The percentage increases in free fatty acid contents of all the spice products packed in the low density polythylene pouches were more in comparison to the cellophane and Al / poly laminate

TABLE 2. SENSORY SCORES FOR AROMA OF EMULSION PREPARED FROM VOLATILE OIL OF CUMIN POWDER

Panelists	CON-S	CG-S
1	8	7
2	7	6
3	8	7
4	6	6
5	7	6
6	7	6
7	8	7
8	7	5
9	6	5
10	8	7
Mean	7.2	6.3

CD at 5% level of significance = 0.51

Two means differ significantly at 5% level

TABLE 3. EFFECT OF VARIOUS STORAGE CONDITIONS AND PACKAGING MATERIALS ON MOISTURE CONTENTS, PLATE COUNTS AND FREE FATTY ACIDS OF BOTH CONTROL AND COLD GROUND SAMPLES

Types of packaging	Storage conditions	Moisture contents, %	Total plate counts, cfu/g	FFA, % oleic acid
CON-S 25°C 90% RH				
Polyethylene		12.41	151 x 10^4	0.16
Cellophane		12.10	136 x 10^4	0.15
Al + Poly		11.16	110 x 10^4	0.13
CG-S 25°C / 90% RH				
Polyethylene		12.21	75 x 10^4	0.14
Cellophane		11.71	68 x 10^4	0.13
Al + Poly		11.52	56 x 10^4	0.12
CON-S 37°C / 70% RH				
Polyethylene		12.10	148 x 10^4	0.17
Cellophane		11.91	131 x 10^4	0.15
Al + Poly		11.52	92 x 10^4	0.14
CG-S 37°C / 70% RH				
Polyethylene		11.89	70 x 10^4	0.16
Cellophane		11.60	62 x 10^4	0.14
Al + Poly		11.31	48 x 10^4	0.13

pouches irrespective of the storage conditions. There was increase in free fatty acid content (45.45 to 54.54%) of CON-S samples packed in the polyethylene pouches during storage. However, increase in free fatty acid contents of CG-S sample was less (29.3 to 45.32%), when stored at 25°C / 90% RH and 37°C / 70% RH, respectively (Table 3). The development of free fatty acids during storage of all oil-bearing materials including spices, assume greater significance in terms of their sensory quality.

Microbial loads of the cumin powder packed in the various packaging materials increased, when stored for 4 months at 25°C / 90% RH and 37°C / 70% RH. The percent increase in the microbial load of the cumin powder stored at 37°C / 90% RH and packed in the Al / poly laminate pouches was less

TABLE 4. SHELF LIFE OF GROUND CUMIN PACKED IN VARIOUS PACKAGING MATERIALS AND STORAGE CONDITIONS

	Storage conditions			
	25°C / 90% RH Shelf life, days		37°C / 70% RH Shelf life, days	
Types of packaging	CON-S	CG-S	CON-S	CG-S
Low density polyethylene	49	60	56	70
Cellophane	57	77	63	84
Al/Polyethylene	84	98	91	119

(Table 3). The percentage increases in the moisture contents of all the ground spice samples packed in low density polyethylene and stored at 25°C / 90% RH, were more and this could explain the increase in the microbial load of the samples packed in the low density polyethylene pouches. Al / poly laminate film has excellent barrier properties against gases and water vapour (Kumar and Anandaswamy 1974) and it could be used for packaging of cold ground cumin powder. Shelf life periods of all the samples packed in various packaging materials and stored at 25°C / 90% RH and 37°C / 70% RH were determined and presented in Table 4. The shelf life periods of CG-S samples of cumin powder packed in low density polyethylene, cellophane and Al / poly laminate pouches were 56, 77 and 98 days, respectively at 25°C / 90%, while these were 70, 89 and 119 days, respectively at 37°C / 70% RH. The shelf life period of the CON-S cumin powder packed in Al / poly laminate and stored at high temperature and low relative humidity (37°C / 70% RH) was 91 days.

Water plays a very important role in the storage of fresh, frozen and dried foods. It acts as a solvent for chemical, microbiological and enzymatic reactions. Ground cumin stored in high relative humidity would undergo various spoilage reactions and impair its sensory attributes.

It may be concluded that grinding of cumin at chilled temperature not only increased the volatile oil content of the products but also it improved the fineness of the particles and sensory qualities. Al / poly pouches and a storage condition of 37°C / 70% RH could be ideal for packaging and storage of cold ground cumin powder.

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RESEARCH NOTE

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Determination of Residual Hexane and Microbiological Status in De-oiled Soybean Meal

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A simple volatization procedure was developed to detect the residual hexane in solvent extracted soy meals with good precision using the gas chromatographic technique. The study showed that this method released and assayed residual hexane up to one ppm. The deoiled soybean meals from different solvent extraction plants were also analyzed for microbial load. These results indicated the presence of *Coliforms, Enterobacteriaceae, Staphylococci, Aerobacter, Bacillus, Aspergillus* and *Mucor* in the isolates.

Keywords : Soybean meal, Residual hexane, Microbial status.

Soybean (*Glycine max* M) with its 40% proteins and 20% oil assumes the most predominant position in solving the food problems created by the ever increasing population in India and other Third world countries. Though a number of soy products are now available in the markets, they are not becoming popular yet. Among them, extruded products like nuggets and oil are extensively used by the Indian consumers. Soybean oil is used after refining. The deoiled cake is mostly exported to other countries, where it is generally used as cattle, swine and poultry feeds. It is finding a place in aqua feeds also (Kikuchi 1999). The soy meal is a rich source of proteins and possibly will combat the protein-energy malnutrition existing in the rural areas of our country. n-hexane is used as a solvent in oil extraction from the soybean but even after its recovery through disolventization systems, a little quantity of n-hexane remains in it. This may cause various physiological disorders in humans. The literature reveals that the workers when exposed to n-hexane suffer from nervous disorders. The United States, as per the Clean Air Amendment Act of 1990, has listed n-hexane among 189 hazardous air pollutants (Johnson and Lusas 1983). However, the effect of residual hexane present in the meal on human health is not well documented yet. As per Bureau of Indian Standards (IS:7336, 1975), residual hexane should not be more than 170 ppm in the meal, while International Standard Organization permits 50 ppm only. As on hand, no information is available on how these limits in the meal were fixed. Besides, there is no simple and convenient method to detect its presence. Since the meal is high in proteins and also poorly handled, it is necessary that the meal should comply with the prescribed microbiological quality. Hence, the present investigation was carried out to establish a simple procedure to detect residual hexane and analyze the microbial load of deoiled cakes from some solvent extractiorl plants in Madhya Pradesh.

De-oiled cakes using the standard techniques were collected afresh from five different solvent extraction plants. These meals have been desolventised and used in the present studies. They were analyzed for microbial load as per the BIS procedures (IS:5401, 1969 a; IS:5403, 1969 b; IS:5887-II, 1976). The chemicals used were of analytical grade. The experiments were carried out in triplicate and the average values are computed.

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Determination of residual hexane : Two layers of Whatman No. 40 filter paper cut to ID of specially designed bottle (Fig 1) were placed for uniform distribution of water (Wan et al. 1977). It had a volume of 150 ml. The filter papers were first wetted and then, a two gram meal sample was placed in the bottle.

This bottle was immediately sealed with the *Bakelite* container cap and placed in a thermally equilibrated water bath kept at 85°C for 6 h. Later, it was allowed to cool to room temperature gradually. Thus, a 20 µl gas sample was taken from the headspace by using an air tight syringe and injected into a Gas Chromatograph (Toshniwal Bros., India Chemito Model). Chromosorb-30 glass column from Toshniwal Bros., Mumbai, India was used. Nitrogen was used as a carrier gas at the rate of 30 ml/min. The detector used was FID with 75°C-250°C programmed temperature with increment of 2°C/min in the column temperature. The temperatures of the column, detector and injector were set at 80°C, 150°C and 150°C, respectively.

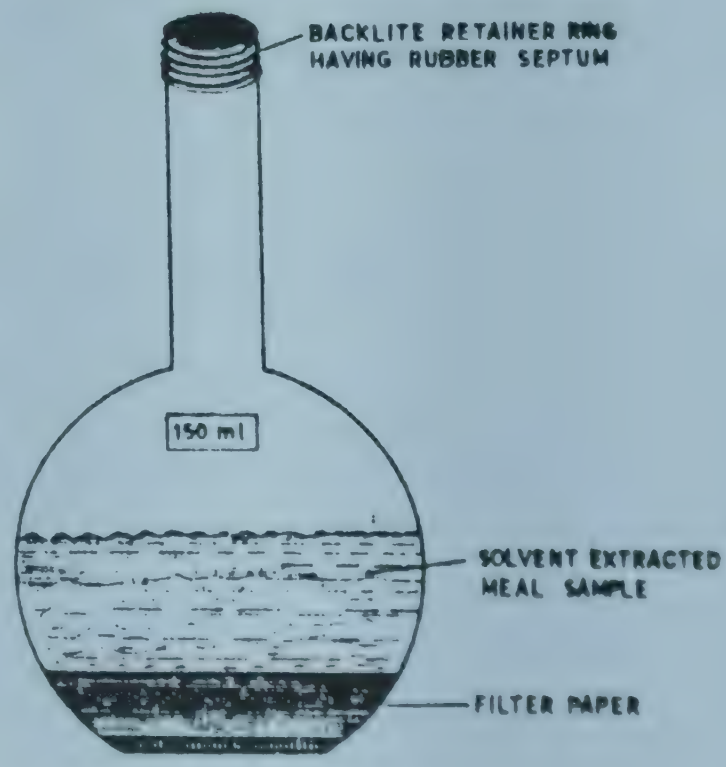

Fig. 1. A cross sectional view of the glass apparatus used in the volatization method

Preparation of calibration curve : Calibration curve of n-hexane was plotted using a known quantity of pure hexane in two grams of soy meal placed in the bottle and the same procedure was followed as described earlier. Fig. 2 shows the typical gas chromatograph of a n-hexane available commercially and hexane extracted from soy meals using the present procedure. The calibration curve is shown in Fig. 3. About 0.5 ml of water was used as the optimum level for a two gram sample as suggested by Wan et al (1977).

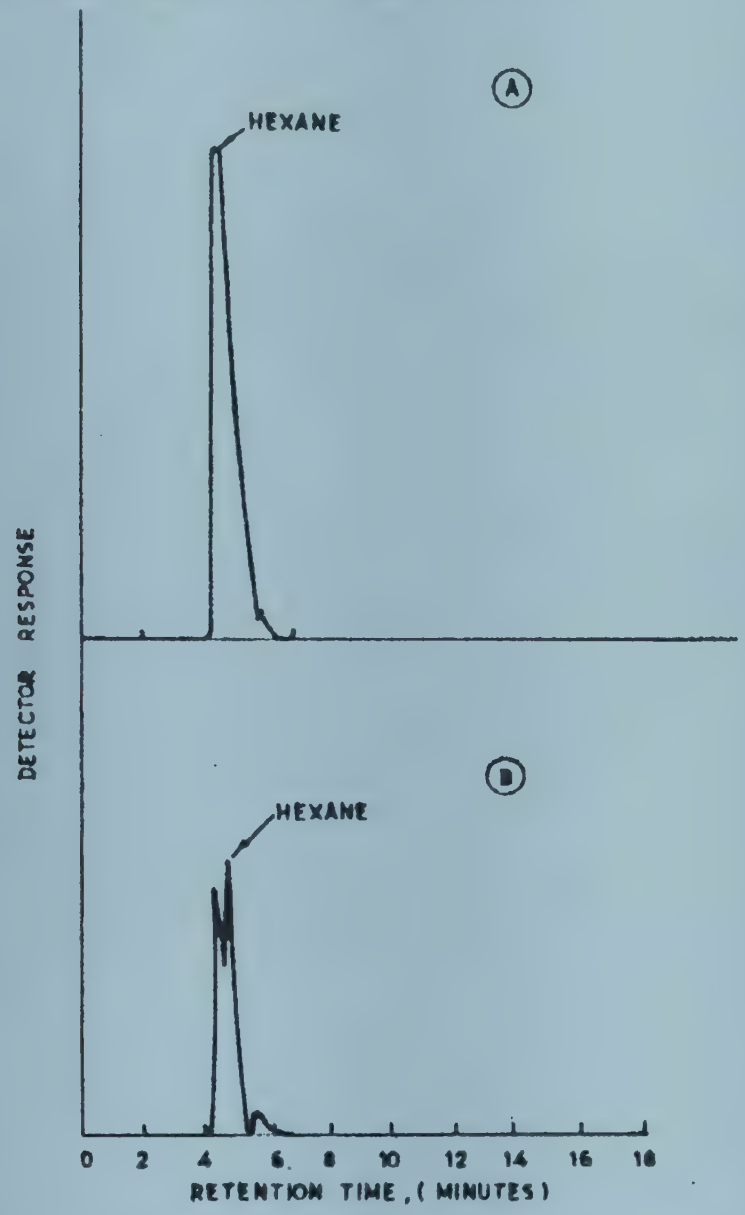

Fig. 2. Gas chromatogram of a commercial hexane (A) and a hexane extracted from soymeal (B) by this method.

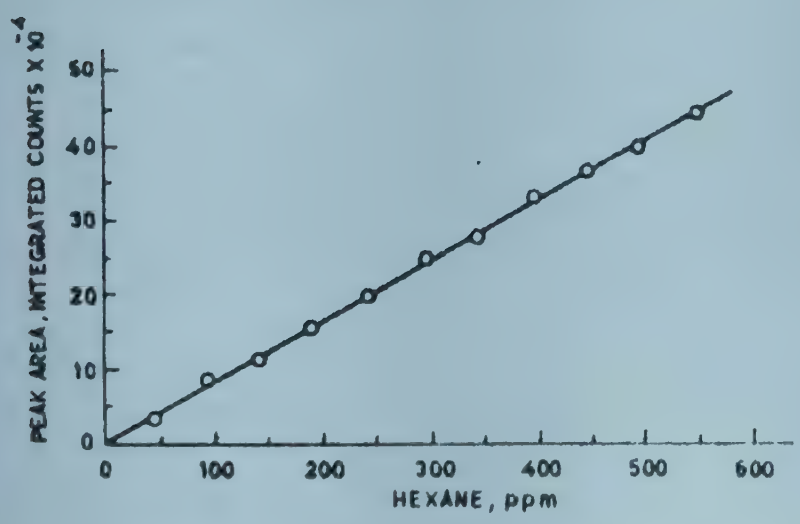

Fig. 3. Calibration curve of hexane

Estimation of residual hexane in the samples : The meal samples collected from five different solvent extraction plants were analyzed by this method and the results are given in Table 1. The data indicate that these samples contain 35-77 ppm hexane, which are within the permissible limits as prescribed by the BIS. The results are quite reproducible even at higher levels of hexane. Thus, this method can be conveniently used to detect the residual hexane in meal with good reproducibility using the Gas Chromatograph and comparable with the results obtained through other methods. The method is very simple to follow. The equipments used are commonly available in any quality testing laboratory. BIS IS:1167 (1986) describes the method for determination of residual solvent in oilseed flour and meals by modified Pensky-Martens' Closed Tester. The assembly of the apparatus is quite complicated. Thus, the present method is simple and reproducible. It covers a wide range of residual hexane in meal.

Assessment of microbial load : The detailed microbial data of different deoiled cakes are presented in Table 2. The data indicate the presence of both bacteria and mold counts. Major bacterial isolates were *Coliforms, Enterobacteriaceae, Staphylococci, Aerobacter* and *Alternaria*. The molds were mostly *Aspergillus* and *Mucor.* No *Salmonella* was detected in these samples. The fungal contamination was more than the bacterial load. As per the Bureau of Indian Standards, the soy-based products should not contain more than 10 numbers of *Coliforms* per gram samples. But the samples A and C had 0.4 x 10^4 and 0.7 x 10^4 colonies, which were highly objectionable. The maximum permissible bacterial count was 50000 nos/gram sample. Most of the samples were with in the limits. However, the bacteria like *Enterobacteria* and *Staphylococcus* are not

TABLE 1. RESIDUAL HEXANE IN THE SOLVENT EXTRACTED SOY MEALS

Sample details	No. of replicates	Residual hexane, ppm	Average deviation, ppm	% deviation, ppm
A	4	76.5	1.2	1.6
B	6	40.3	0.6	1.5
C	4	48.0	0.8	1.7
D	3	35.2	0.5	1.4
E	5	68.0	1.0	1.5

TABLE 2. BACTERIAL AND FUNGAL COUNTS OF DIFFERENT DEOILED SOYBEAN CAKES

Sample	Bacterial		Fungal	
	cfu* (X10^4)	Isolates	cfu* (X10^4)	Isolates
A	0.4	*E. freundi*	0.3	*Aspergillus* spp
B	0.4	*Bacillus megatarium* *Alternaria* spp	11.4	*Aspergillus* spp
C	0.7	*E. freundi* *Aerobacter aerogenes*	–	–
D	1.2	*Enterobacter* spp	7.2	*Aspergillus* spp
E	0.5	*Staphylococcus* spp	8.0	*Aspergillus* spp *Mucor* spp

* Colony forming units/g sample

desirable. With regard to fungal load, the standards did not quantify any number. But the de-oiled cakes had fungal counts in the range of 0.3 x 10^4 and thus the results revealed that any of these de-oiled soy cakes were not fit for either human or cattle consumption. Hence, there is a need to create proper sanitary conditions in the process of making de-oiled cakes. The hygienic conditions in most of the solvent extraction plants is also inadequate. The studies thus indicated the necessity of improving the processing conditions to get de-oiled cakes of edible grade free from hazardous microflora.

It may be concluded that the quality of the de-oiled soy cake needs to be monitored in terms of the residual solvent and the microbial load for its further utilization.

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RESEARCH NOTE

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Pearling Treatment of Discoloured Sorghum Grains

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A simple process was standardized to obtain about 80% clear, white edible-grade product from mould-infested and discoloured sorghum grains. The discoloured grains were suspended in 40% NaCl (sp.gr. 1.25) to separate heavily infested and light weight grains. The sinkers were dried and pearled on an abrasive polisher at 650 rpm for 5 min. The pearling treatment removed completely the surface discoloration and aflatoxins, while lowering the contents of fibre by 15.6% and polyphenols by 20.6%. The pan breads from meal of discoloured grains were unacceptable, while those prepared from pearled product were found acceptable.

Keywords : Discoloured sorghum, Pearling, Aflatoxins, Pan breads.

In India, the kharif-grown sorghum crop is often damaged in late rains during grain development. The humid and wet conditions favour mould infestation and black discoloration in the matured grains. The extent and severity of surface blackening vary greatly depending on cultivar, grain-filling stage, type of mould etc. The infected grains may show a negligible to complete blackened surface. Within a same lot, certain proportions of grains remain unaffected, some suffer from only surface blackening, while some are heavily infested and totally damaged. The normal mean thousand grain weight (28.2 g) and mean grain hardness (7.2) have been found to decrease by about 12% and 40%, respectively in completely discoloured grains of 10 cultivars (Deshmukh 1999). The spices of *Fusarium, Alternaria, Drechslera* and *Curularia, Aspergillus* have been identified from blackened grains (Narayana et al. 1980). Such grains are unacceptable for human consumption mainly due to discolouration. All discoloured grains may not contain aflatoxin. Both growers and procurement agencies have to bear heavy economic losses. Such a produce possesses a limited shelf life and needs to be processed immediately, if it is to be utilized either for feed or food purpose.

Such grains can be cleaned to separate totally the damaged light weight fraction and the remaining surface blackened grains can be processed by pearling to obtain a white product. Alkali treatment of such discoloured sorghum has been reported to produce about 85% clean product, (Darade et al. 1999). However, the treatment is expensive and inconvenient. Hence, in this investigation, an attempt was made to standardize a cleaning and physical pearling treatment of such grains and study the changes in nutritional composition and shelf life of the pearled product.

The blackened samples of 10 kharif-grown sorghum cultivars of 1998 harvest with about 12% moisture were cleaned to remove debris and adhering glumes by light brushing on an abrasive polisher. The heavily infested and damaged grains were separated by the following two ways: One kg grains of each cultivars were subjected to Damas gravity separator. The medium and heavy weight fractions were combined (79.1%). In order to develop an alternative procedure that could be adapted at domestic level, the mean specific gravity of normal white grains of all 10 cultivars was calculated in quadruplicate (1.25) and NaCl solution (40% w/v), with specific gravity about 1.25 was used to separate the heavily infested grains. One kg grains of each cultivar were suspended in 40% NaCl solution (1:2 w/v) and the floater grains were removed (23.3%), while the sinker grains were recovered (76.7%) and dried at 40°C for 2 h in a laboratory oven to about 10% moisture. The process required a few minutes when the sinkers absorbed not more than 1% additional moisture. The sinkers were dried directly without washing with water. The NaCl on grain surface was subsequently removed during the pearling treatment of these grains. The recovery of sinkers was, comparable with the grain fraction obtained by gravity separator.

The discoloured grains (250 g) were pearled on Lab-model abrasive rice polisher INDOSAW at 650 rpm for 2 to 5 min or at 1050 rpm for 1 to 3 min. On the basis of complete whiteness and minimum dry matter loss, pearling of discoloured grains at 650 rpm for 5 min was found to be optimum. The discoloured sinkers of 10 cultivars (250 g) were then subjected to pearling at 650 rpm for 5 min. The total recovery of white product and proportion of whole pearled grains in this were determined. The process is briefly depicted in Fig. 1.

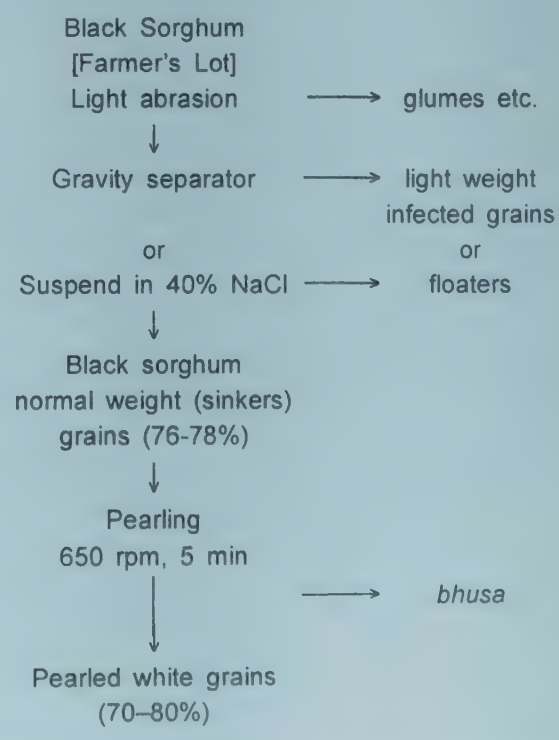

Fig. 1. Pearling process for black sorghum with abrasive rice polisher

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The original discolored grains and the pearled products were analyzed for crude proteins, crude fat, acid value, total ash, fibre, aflatoxin B_1 (AOAC 1990), reducing and total sugars (Nelson 1944) and polyphenols (Swain and Hillis 1959). The discoloured grains and the pearled product as well as their meals produced in a laboratory mill were stored in plastic boxes under controlled conditions at 30°C and 80% RH up to 30 days. The samples were analyzed for changes in proteins, fat, acid value of oil and total sugars. Pan breads *(roti)* were prepared of 50 g meal each by the traditional method at 0 and 30 days of storage and evaluated for appearance, taste, flavour and texture by a panel of semi-trained judges using a 10-point scale. The means of scores were used to determine *roti* acceptability.

TABLE 1. PROCESSING CHARACTERISTICS OF DISCOLOURED SORGHUM GRAINS

Cultivar	Discoloured grains		Pearled product	
	Gravity separation, recovery, %	Sinkers in 40% NaCl recovery, %	Total recovery, %	% whole grains
'SPV-1293'	60.0	56.9	66.6	46.7
'SPV-1333'	80.7	77.7	71.7	57.3
'SPV-1403'	83.4	81.4	88.9	89.5
'SPV-1384'	87.9	84.8	88.1	95.1
'SPV-1385'	76.6	73.8	71.9	51.3
'SPV-96'	74.4	72.3	80.9	73.1
'CSV-13'	72.2	70.1	75.7	56.8
'CSH-1'	82.4	80.4	76.8	67.8
'CSH-5'	98.1	96.2	81.3	77.6
'CSH-9	75.3	73.4	90.5	92.4
Mean	79.1	76.7	79.2	70.8
S.D.	7.2	6.9	8.2	7.8

TABLE 2. EFFECTS OF PEARLING OF DISCOLOURED SORGHUM GRAINS ON NUTRITIONAL COMPOSITION AND ALFATOXIN B_1* (mean of 10 cultivars)

Parameter, %	Discoloured grains	Pearled product	Loss, %
Crude proteins,	8.0 ± 0.23	7.5 ± 0.21	5.6
Crude fat	2.3 ± 0.04	1.7 ± 0.04	24.1
Total ash	1.2 ± 0.03	1.1 ± 0.03	8.4
Crude fibre	1.8 ± 0.01	1.6 ± 0.01	15.6
Reducing sugars	0.25 ± 0.05	0.18 ± 0.04	30.8
Non-reducing sugars	1.5 ± 0.02	1.4 ± 0.02	9.5
Polyphenols	0.18 ± 0.01	0.14 ± 0.01	20.6
Aflatoxin, B_1 µg/kg	100.0 ± 10.28	Nil	100.0

*composite sample of 10 cultivars was analyzed for aflatoxin B_1 content.

The mean yield of medium to heavy weight grain fraction was 79.1% with gravity separator, while it was 76.7% for sinkers in 40% NaCl solution (Table 1). These results have indicated that sodium chloride solution with sp.gr. of 1.25 can be conveniently used to separate heavily infested, light weight grains at domestic level. The pearling of sinkers at 650 rpm for 5 min produced an average of 79.2% white product. The pearled product contained 70.8% of whole grains. The cultivars exhibited market variations for both the yields of sinkers and pearled product. A pearling treatment was found to lower the contents of nutritional constituents, particularly fat (24.1%), fibre (15.6%) and polyphenols (20.6%) with a complete elimination of aflatoxin B_1 (Table 2). These results have indicated that the discoloured sorghum can be economically processed into an edible grade product with about 80% recovery by abrasive pearling treatment.

The nutritional composition and sensory evaluation of *roti* prepared from the discoloured grains and their meals after 30 days of storage were found to deteriorate and exhibit unacceptable quality. While the pearled product or its meal showed relatively better shelf life and excellent *roti* quality even after 30 days of storage of meal/flour at ambient conditions (Table 3).

Several reports are available on dry partial pearling of normal white sorghum (Raghavendra Rao and Desikachar, 1964; Hahn 1969; Chandrashekar and Desikachar, 1984; Varadharajan et al. 1989). The principal objective of these studies was to remove the blackened surface layer completely and obtain a white product. The dry matter and nutrient losses are obvious due to scouring of surface layers. Such losses may be of higher magnitude in pearling of discoloured grains because a near complete pearling is essential to prevent discolouration. A dry matter loss of about 20-25% can, however, be offset by recovering nearly 75 to 80% of the edible-grade pearled product. The losses due to discolouration and during subsequent pearling will vary due to degree of infestation and type of cultivar. The technology described may also be useful at village level, if mini-abrasive rice polishers are installed alongwith traditional flour mills. The experiments to develop a suitable technology for obtaining a value-added product with extended shelf life such as starch from discoloured sorghum are in progress.

TABLE 3 EFFECTS OF PEARLING AND STORAGE OF DISCOLOURED SORGHUM ON NUTRITIONAL COMPOSITION AND *ROTI* ACCEPTABILITY

Product	Nutritional composition % loss (–) or gain (+) after 30 day storage*				*Roti* acceptability*	
	Crude proteins	Crude fat	Acid value	Total sugars	0 day meal	30 day meal
Grains - discoloured	– 9.0	+ 24.1	+ 4.7	– 10.0	5.0	4.6
Grains - pearled	– 5.5	+ 10.0	+ 2.6	– 5.9	7.6	7.1
Meal - discoloured	– 8.6	+ 6.8	+ 3.4	– 10.0	5.0	5.0
Meal - pearled	– 4.1	+ 10.0	+ 1.3	– 5.9	7.6	7.6

* The grains and meals stored in plastic boxes at 30°C, 80% RH in humidity chamber and analyzed at 0 and 30 days of storage

* The fresh *rotis* were evaluated for appearance, flavour, taste and texture properties by a panel of 5 judges on 10 point scale and mean values are considered for overall acceptability.

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RESEARCH NOTE

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Studies on the Factors Affecting the Physico-chemical and Organoleptic Properties of Kinnow Juice

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Four extraction methods, i.e., screw type extractor, superfine pulper, hydraulic press and rosing machine; three pretreatments i.e., lye treatment of segments, ethrel treatment and chilling of fruit and different levels of additives such as sugar, salt and *chatmasala* were used for the study. Juices from screw type extractor and superfine pulper were adjudged the first and second by the panelists respectively. Lye treatment of segments resulted in lowering of total soluble solids, total solids, total sugars, ash and acid contents of juice but improved the flavour scores significantly. Ethrel treatment of fruit resulted in increases in total soluble solids, reducing sugars, total sugars and decrease in pectin content. Chilling of fruit before extraction of juice had no significant effect on the physico-chemical and organoleptic properties of juice. The juice having 16° Brix (with added sugar), 0.2% common salt or 0.2% *chatmasala* got the highest organoleptic scores. Total soluble solids, total solids, pH, acid, ash, pectin and salt contents remained unchanged. The reducing sugars and non-enzymatic browning increased significantly, whereas ascorbic acid decreased significantly during storage. Juices were found organoleptically acceptable up to six months of storage at room temperature (15-40°C).

Keywords : Kinnow juice, Extraction method, Pre-treatment, Additive, Storage, Physico-chemical properties, Organoleptic properties.

Kinnow mandarin orange is an important commercial fruit of Punjab and adjoining States. Fully ripe fruit has a bright attractive orange colour. The fruit is very rich in juice and liked very much by consumers. The post-harvest shelf life of fruit at room temperature is very limited (Jawanda and Singh 1973) and shelf life can be extended to a maximum period up to 45 days under refrigerated storage conditions. In view of its limited shelf life, the fruit must be processed to extend its availability period and also to minimize the glut in the market in its peak season of production. Kinnow juice turns bitter during extraction and heat processing and is not liked much by majority of consumers. A few studies have been conducted on the effect of pH (Ranote and Bains 1982), lye treatment (Sandhu et al. 1990) and extraction methods (Lotha and Khurdia 1994) on kinnow juice. To popularise kinnow juice among masses, it is necessary to develop suitable technology, which should be commercially feasible to process this highly nutritious and juicy fruit. The present investigation was undertaken to study the effect of various extraction methods, pre-treatments and additives on the physico-chemical and organoleptic qualities of kinnow juice on storage.

Kinnow mandarin fruit about 20 kg for each experiment was utilized for conducting the study as follows:- (i) Four extraction methods i.e. superfine pulper, screw type extractor, hydraulic press and rosing machine were used for extraction of juice, (ii) Three pre-treatments i.e. lye treatment (1% sodium hydroxide at 82-83°C for 30 sec, followed by neutralization) of segments (Sandhu et al. 1990), dipping the fruit in 400 ppm ethrel for few seconds and holding the fruit at 20°C (Maier et al. 1973), and chilling to 3-5°C of fruits overnight before extraction of the juice and (iii) Three additives i.e., sugar to adjust total soluble solids from 13 to 16° Brix, common salt 0.2 to 0.8% and *chatmasala* 0.2 to 0.6% were added to juice to optimise their levels.

*Corresponding Author

Juice with additive was heated to 80-82°C and hot-filled in bottles of 200 ml capacity, crown-corked and processed in boiling water for 20 min, followed by immediate cooling in running cold water. The bottled juice was stored under ambient conditions (15-40°C) and analysed at 30 day intervals for 6 months. The physico-chemical parameters including total soluble solids (TSS), total solids, titratable acidity (as citric acid anhydrous), pH, reducing and total sugars, ascorbic acid, ash, pectin (as calcium pectate) and salt were determined by AOAC (1984) methods and non-enzymatic browning was determined by the method described by Ranganna (1977). In order to find out the consumer preference of juice extracted by different methods, the ranking evaluation test was used, while the extraction methods, pre-treatments, additives and storage on the organoleptic quality of juice was studied on a 9-point Hedonic scale (Amerine et al. 1965). The organoleptic panel consisted of ten semi-trained panelists.

Effect on physico-chemical properties : The results of juice recovery and composition as affected by methods of extraction are given in Table 1. Superfine pulper gave the highest juice recovery (49.78%), while rosing machine gave the lowest juice recovery (32.77%) among the four methods used. The results indicated that the methods of extraction resulted in greater variation in the juice recovery as compared to values reported (36.36 to 45.50%) by Lotha and Khurdia (1994) using different types of juice extractors.

Juice extracted by different methods had minor variation in composition, but significant at $P<0.05$ except pectin and ash contents of juice extracted by hydraulic press, which recorded exceptionally low values for these components. The alteration in composition of juice may be attributed to variations in juice recovery and incorporation of fruit components into juice to variable extent by different machines. The juice extracted by hydraulic press gave clear juice, filtered through canvas cloth excluding all suspended particles, resulting in the lowest pectin and ash contents among all the methods used

TABLE 1. EFFECT OF METHOD OF EXTRACTION ON COMPOSITION OF KINNOW JUICE

Parameter	Superfine pulper	Screw type extractor	Hydraulic press	Rosing machine	CD (5%)
Juice recovery,%	49.78	46.50	38.41	32.77	-
TSS, °Brix	10.36	10.40	10.17	10.70	0.074
Total solids, %	11.57	11.52	10.78	12.12	0.040
Acidity, %	0.45	0.47	0.43	0.45	NS
pH	4.13	4.10	4.14	4.15	NS
Reducing sugars,%	3.87	3.98	4.00	3.81	0.065
Total sugars, %	7.52	7.75	7.31	7.72	0.063
Ascorbic acid, mg%	7.01	8.37	8.16	8.81	0.063
Pectin, %	0.28	0.27	0.11	0.28	0.030
Ash, %	0.38	0.41	0.32	0.40	0.028

TABLE 2. EFFECT OF PRE-TREATMENT ON COMPOSITION OF KINNOW JUICE

Parameter	Treatment					
	Lye treatment		Ethrel treatment		Chilling treatment	
	Control	Experimental	Control	Experimental	Control	Experimental
TSS, °Brix	12.10	11.40	11.75	12.40	11.80	11.77
Total solids, %	13.39	12.34	12.97	13.66	13.11	13.07
Acidity, %	0.56	0.50	0.49	0.48	0.46	0.44
pH	3.96	4.08	4.10	4.12	4.17	4.21
Reducing sugars,%	4.18	3.87	3.91	4.17	3.58	3.50
Total sugars, %	8.62	7.87	8.17	8.57	8.21	8.27
Ascorbic acid, mg%	11.73	9.65	12.71	12.50	14.71	14.80
Pectin, %	0.24	0.21	0.21	0.16	0.22	0.21
Ash, %	0.38	0.34	0.46	0.47	0.43	0.44

The juice from pre-treated fruits was extracted by screw type extractor. The composition of juice from different pre-treatments is given in Table 2. Lye treatment of segments resulted in lowering of all components possibly due to leaching during treatment. There was a slight increase in pH, resulting from the lowering of titratable acidity. Ethrel treatment of fruits resulted in increases in total soluble solids, reducing and total sugars, total solids, ash and pH but negligible decreases in acidity and ascorbic acid. The pectin content was affected considerably by ethrel treatment. The increases in values of different components may be due to conversion of complex carbohydrates into simple components due to sensescence by treatment. The decreases in titratable acidity and pectin may be attributed to breakdown of these components by increased respiration rate and activation of pectinase by ethrel treatment. Chilling treatment of fruit was found to have no effect on composition of resultant juice.

The composition of juice extracted by superfine pulper and screw type extractor with the incorporation of different additives is given in Table 3. There was minor alteration in the natural composition of juice with incorporation of additives used and same is given in the corresponding column of the Table at the initial stage.

There were no significant changes in total soluble solids, acidity, total sugars, total solids, pectin, ash and salt contents, whereas ascorbic acid, reducing sugars and optical density of juice changed significantly ($P<0.05$) during six month storage. The loss in ascorbic acid content was found to be more in case of juice extracted by superfine pulper. The increase in the reducing sugars may be due to inversion of non-reducing sugars. Non-enzymatic browning also increased as indicated by the increase in optical density of juice. The extent of browning was more in stored juice from superfine pulper. Both the loss of ascorbic acid and increase in reducing sugars might have contributed to increase in the non-enzymatic browning of juice. The samples having *chatmasala* recorded significant decrease in pH during storage which resulted from increase in acidity of these samples. Increased acidity in these samples could be the result of hydrolysis of some components present in *chatmasala.* Comparison of the two methods revealed that during storage, the juice extracted by screw type extractor had better retention of nutrients.

Effect on organoleptic properties : The order of consumer preference on the basis of overall organoleptic quality of juice from screw type extractor, superfine pulper, hydraulic press and rosing machine was the first, second, third and fourth respectively. The differential preference of juice extracted by different methods showed that the method of extraction affected the organoleptic properties of juice. On the basis of these results, only the first two methods of extraction were selected for further studies and natural juice from these two methods was got evaluated on 9-point Hedonic scale and the results are given in Table 4. There was significant ($P<0.05$) difference in the mean score of flavour from screw type extractor and superfine pulper, while there was no significant difference in the mean scores of appearance and body from two methods. The high preference of juice from screw type extractor was found due to instantaneous pressing of segments directly with minimum crushing of tissues and without much oxidation of juice resulting in less bitterness as compared to superfine pulper. In case of superfine pulper, fruit segments and seeds were crushed by high speed revolving paddlers, which also whipped the air into juice during extraction resulting in lower preference. Bruemmer et al (1973) reported similar causes of lower preference of orange juice.

Among the three pre-treatments used, lye treatment resulted in significant ($P<0.05$) increase in flavour score, which may be attributed to dissolution of albedo portion of segments, containing bitter principles by hot lye treatment. Other treatments were found to improve the organoleptic properties slightly but alterations in appearance, body and flavour mean scores of the juice were found non-significant.

Juice extracted by screw type extractor was used to study the effect of additives on organoleptic properties of juice (Table 5). Initially, TSS of juice was adjusted with sugar from 13° Brix to 16° Brix to find out the effect of increased sugar

TABLE 3. EFFECT OF STORAGE ON COMPOSITION OF KINNOW JUICE FROM SUPER FINE PULPER AND SCREW TYPE EXTRACTOR

Parameter	Natural juice		Juice 16° Brix		Juice 16° Brix and 0.2% salt		Juice 16° Brix and 0.2% Chatmasala	
	Initial	After 6 months	Initial	After 6 months	Initial	After 6 months	Initial	After 6 months
				Super Fine Pulper				
TSS, °Brix	11.40	11.37	16.00	16.05	16.05	16.05	16.00	15.97
Total solids, %	12.50	12.30	17.60	17.65	17.78	17.25	17.70	17.75
Acidity, %	0.36	0.37	0.36	0.37	0.37	0.38	0.36	0.39
pH	4.20	4.18	4.19	4.17	4.18	4.19	4.20	4.08*
*Reducing sugars,%	3.97	6.31	4.28	7.37	4.37	7.89	4.11	4.27
Total sugars, %	8.77	8.65	13.51	13.33	13.43	13.30	13.25	13.10
*Ascorbic acid, mg%	13.68	3.80	13.53	4.81	13.02	3.59	14.56	4.31
Pectin, %	0.25	0.24	0.28	0.26	0.31	0.29	0.30	0.27
Ash, %	0.38	0.37	0.39	0.38	0.43	0.42	0.42	0.41
*OD at 440 nm	0.06	0.15	0.08	0.17	0.07	0.16	0.07	0.17
Salt, %	-	-	-	-	0.18	0.17	0.12	0.11
				Screw Type Ectractor				
TSS, °Brix	10.20	10.25	16.20	16.17	16.00	15.97	15.97	15.97
Total solids, %	11.25	11.30	17.30	17.40	17.71	17.75	16.68	16.90
Acidity, %	0.30	0.30	0.29	0.30	0.29	0.32	0.29	0.33
pH	4.49	4.47	4.56	4.52	4.53	4.43	4.54	4.40*
*Reducing sugars,%	3.14	5.38	3.60	7.14	3.58	7.32	3.62	7.78
Total sugars, %	7.73	7.56	13.27	13.11	12.98	12.82	13.11	12.96
*Ascorbic acid, mg%	9.60	3.02	9.55	3.81	9.08	3.01	9.04	3.56
Pectin, %	0.19	0.18	0.21	0.19	0.24	0.21	0.22	0.19
Ash, %	0.35	0.35	0.36	0.37	0.39	0.37	0.37	0.36
*OD at 440 nm	0.05	0.12	0.08	0.14	0.08	0.14	0.07	0.14
Salt, %	-	-	-	-	0.19	0.18	0.11	0.11

* Significant difference at CD 5% level

TABLE 4 EFFECT OF METHOD OF EXTRACTION AND PRE-TREATMENT ON ORGANOLEPTIC PROPERTIES (MEAN HEDONIC SCORES) OF JUICE

Parameter	Extraction method		Pre-treatment					
			Lye treatment		Ethrel treatment		Chilling treatment	
	Superfine	Screw type extractor	Control	Experimental	Control	Experimental	Control	Experimental
Appearance	7.25	7.50	7.58	7.91	7.00	7.25	8.06	7.75
Body	7.25	7.25	7.58	7.95	7.12	7.75	7.37	7.37
Flavour	4.62	5.87*	6.12	7.12*	6.25	6.45	6.08	6.62

* Significant difference at CD 5% level

concentration on flavour of juice. It was observed that the flavour score increased with increasing levels of TSS and it was highest with 16° Brix. On the basis of this, TSS level of 16° Brix was used to find out the compatibility of salt in juice. The juice with sugar (16° Brix) alone gave lower flavour score when used as control for salt. The addition of 0.2% salt increased the flavour score to highest 8.10 and further increase in concentration of salt lowered the flavour score. Again, juice with 16° Brix was used to find out the effect of *chatmasala* on flavour score. With this again, the control gave lower flavour score, while addition of *chatmasala* at a level of 0.2% gave the highest flavour score of 8.31 and further increase in concentration decreased the score. Finally, on the basis of consumer preference, the best combination was found to be the juice with 16° Brix and 0.2% *chatmasala* for highest flavour score. It was observed that the flavour score of control varied with the additives used. When sugar levels were increased, the flavour score increased due to the balancing effect of Brix/acid ratio.

Organoleptic properties of juice were affected by storage depending upon the method of extraction and additives used (Table 6). Significant reduction took place in the appearance score only in juice with 16° Brix from superfine pulper, while flavour scores were affected significantly in all the three samples stored with 16° Brix and 0.2% salt and 16° Brix and 0.2% *chatmasala* from superfine pulper. No significant changes

TABLE 5. EFFECT OF ADDITIVE ON FLAVOUR (MEAN HEDONIC SCORE) OF JUICE

Sugar levels	Score	Sugar and salt levels	Score	Sugar and *chatmasala* levels	Score
13° Brix	6.40	16° Brix and 0%	7.35	16° Brix and 0%	7.20
14° Brix	7.30	16° Brix and 0.2%	8.10	16° Brix and 0.2%	8.30
15° Brix	7.80	16° Brix and 0.4%	7.20	16° Brix and 0.4%	7.60
16° Brix	8.10	16° Brix and 0.6%	6.85	16° Brix and 0.6%	6.90
-	-	16° Brix and 0.8%	6.00	-	-
CD (5%)	0.724	-	0.926	-	0.813

TABLE 6. EFFECT OF STORAGE ON ORGANOLEPTIC PROPERTIES (MEAN HEDONIC SCORE) OF JUICE

Sample	Appearance				Body				Flavour			
	Superfine pulper		Screw type extractor		Superfine pulper		Screw type extractor		Superfine pulper		Screw type extractor	
	Initial	After 6 months	Initial	After 6 months	Initial	After 6 months	Initial	After 6 months	Initial	After 6 months	Initial	After 6 months
Natural Juice	7.87	7.00	7.72	7.25	7.25	6.75	7.50	6.87	5.87	4.87	6.50	5.75
Juice 16° Brix	7.87	6.50*	8.25	7.37	7.75	7.12	8.00	7.25	7.50	5.87*	7.75	7.25
Juice 16° Brix and 0.2% salt	7.75	7.25	8.12	7.62	8.00	7.12	7.87	7.37	7.75	6.12*	8.25	7.50
Juice 16° Brix and 0.2% *Chatmasala*	7.87	7.25	7.62	7.37	7.62	7.25	8.00	7.37	7.62	6.00*	8.12	7.25

* Significant difference at CD 5% level

($P<0.05$) were found in organoleptic qualities of any of the stored juice samples from screw type extractor.

All the samples were found acceptable up to 6 months of storage. Overall qualities including appearance, body and flavour scores were, however, better in the juice extracted by screw type extractor than that extracted by superfine pulper after 6 months of storage.

From the present study, it was concluded that physio-chemical and organoleptic properties were affected by methods of extraction, pre-treatment, incorporation of additives and storage period. The screw type extractor was found to be the best among the extraction methods. Lye treatment and addition of sugar, salt and *chatmasala* improved the organoleptic qualities significantly. The juice extracted by screw type extractor retained better quality during storage over superfine pulper.

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RESEARCH NOTE

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Effect of Fermentation on Available Lysine Content of Dried Bovine Liver

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Dehydrated bovine liver was prepared by drying it after lactic acid fermentation. The liver was pasteurized and fermented for 56 h at room temperature and dehydrated. The dried liver contained significantly ($P<0.05$) higher amount of biologically available lysine as compared to the control.

Keywords : Bovine liver, Lactic acid fermentation, Available lysine, Dried product.

Liver is an excellent source of proteins, essential amino acids, vitamin A and other vitamins and minerals (Anderson 1988; Souci et al. 1989/90; Scotter et al. 1992). It is liked for its delicate flavour. Liver pate, liver sausage, liver extract and liver stock are industrially prepared from the liver. These are highly perishable due to their high moisture and nutrient contents. Microbiology of liver has been reviewed by Gill (1988). Different preservation methods may be applied to preserve liver. Dried liver may be used in the development of nutritious food products.

Bovine liver contains 1.6-5.8% carbohydrates (Anderson 1988; Souci et al. 1989/90). Analytical data about the low molecular weight sugars in bovine liver are, however, not known. Cooking process affects the nutritive quality of liver, as amino acids and glucose contained in the liver participate in non-enzymatic browning reactions. Maillard reaction is believed to be responsible for the brown discoloration and bitter taste of canned liver sausage (Jaud 1993). Bovine liver is an excellent source of essential amino acid, lysine, which owing to its highly reactive amino group easily takes part in Maillard type of reactions and thwarts its physiological functions (Carpenter 1973; Hurell and Carpenter 1981; Mauron 1990).

The objective of the present work was to study the effectiveness of lactic acid fermentation on the removal of glucose from bovine liver and to evaluate the effect of this treatment on lysine retention in the dried liver by estimating available lysine.

Lobus sinister laternlis from five fresh beef liver was washed and made in 1-1.5 cm thick slices. The liver slices were kept immersed in boiling water with an internal temperature of 70°C for 15 min. After cooling and draining, the slices were minced twice through a 2-mm plate in a meat mincer and mixed thoroughly. The sample was analysed for glucose content. The prepared material was divided into two portions; to the first portion, 0.1% by weight of lyophilised cells of *Lactobacillus curvatus* was thoroughly mixed in a Hobart mixer. The inoculated liver was filled in vaccumiseable plastic bag, sealed under vacuum and allowed to ferment at room temperature (20°C) for 26 h. A 13% warm solution of sodium bicarbonate was then added to the fermented liver to raise the pH to almost the initial value. The process of fermentation was further continued for another 30 h. The pH of the fermenting liver was noted at 0, 24, 26, 50 and 56 h intervals. The fermented liver was then pasteurized by immersing the liver-bag in boiling water for 15 min (internal temperature 70°C). After cooling and draining, it was ready for the determination of glucose. The second portion of the live did not receive the fermentation treatment. Both fermented and unfermented liver samples were thinly spread on trays and dried in a mechanical drier at 50°C. The dried liver was analysed for moisture, available lysine and colour properties.

Available lysine was determined in dried liver according to Rabasseda et al (1988). pH was measured with the help of an electrode (Type U 402-S 7, Fa. Ingold, Frankfurt/Main) and Microprocessor pH meter pH 537. Glucose was determined by the enzymatic method of Bohringer (Anon 1989). Colour measurement was done with Chromameter CR-200 (Minolta, Japan). The sample of dried and ground liver filled and levelled in petri dishes was illuminated with the light source D 65 and L^*, a^* and b^* values were measured at 0° viewing angle (Jaud 1993). The results were statistically treated by using Paired Difference test (Jellinek 1981).

Cooking is an important method of food processing. Maillard reaction rapidly proceeds in heat processed and concentrated foods. The effect of Maillard reaction on protein quality has been reviewed (Mauron 1990). Ames (1990) summarized the methods of controlling it in food processing. Some of the suggested methods such as elimination of glucose by glucose oxidase in the preparation of egg powder are adopted by industries. Henricksen et al (1955) found improved shelf life of dehydrated pork after removing glucose from meat by treating it with yeast prior to drying. Westphal et al (1986) reported 10% loss of lysine in cooked pork liver mainly due to Maillard reaction.

Table 1 shows the changes in pH of liver during fermentation and the initial and final concentration of glucose in the liver. The mean initial glucose content of the five pasteurized livers was found to be 2.13%, the range being 1.44 to 2.89%. The pH values of all test livers fell rapidly until 24 h of fermentation, after which the rate of pH fall was low, and after 26 h, the fermentation came almost to a grinding halt. It was found that the liver at this stage of fermentation still contained 40-50% of the total concentration. The fermentation could further proceed only after raising the pH

TABLE 1. CHANGES IN pH AND GLUCOSE CONCENTRATION OF LIVER DURING FERMENTATION (n=5)

Fermentation time, h	pH*	Glucose concentration, g/100g
0	6.69 (6.41-6.87)	2.13 (1.144-2.89
24	4.91 (4.49-5.36)	
26	4.80 (4.46-5.19)	
26*	6.46 (6.12-6.77)	
50	5.14 (4.77-5.66)	
56	5.12 (4.68-5.70)	0.03 (0.01-0.09)

*pH value after the addition of sodium bicarbonate

by the addition of sodium bicarbonate. It can be seen from Table 1 that nearly 97-99% of the total glucose contained in the liver was removed in 56 h of fermentation. The colour values of L*(lightness), a*(red), b*(yellow) of test (fermented) and control (unfermented) liver were found to be 76.06 ± 1.16, 1.42 ± 0.42, 13.7 ± 1.04 and 71.18 ± 2.15, 2.40 ± 0.41, 15.28 ± 0.8, respectively. Dried fermented liver showed lighter colour compared to the control. L* value of the test liver was found to be higher than the control counterpart, whereas a* and b* values were lower ($P<0.05$). The contents of available lysine in the glucose-free dried liver and the dry liver, which contained glucose, while drying, were significantly different ($P<0.05$). The test (fermented liver) and the control (unfermented liver) samples contained 4.54 ± 0.02 % and 4.26 ± 0.15% of available lysine on dry weight basis, respectively. These results clearly indicated the occurrence of Maillard reaction during drying of the bovine liver and confirm the loss of lysine in the dried liver. It showed that lactic acid fermentation could remove glucose from the liver and thus loss of lysine could be prevented. Maillard reaction can damage even other amino acids like cystine. However, there are contradictory reports (Westphal et al. 1986; Mauron 1990; Ames 1990).

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RESEARCH NOTE

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Phenolic Antioxidants of Some Common Pulses

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Fourteen species of common pulses were screened for flavonoids and phenolic acids. Flavonoids were present in 11 pulses. Flavones and glycoflavones were present in three and flavonols in the rest. *Vicia faba* is rich in anthocyanins. Phenolic acids are omnipresent. The role of these phenolic compounds in human system is discussed.

Keywords : Antioxidants, Pulses, Flavonoids, Phenolic acids.

The minor constituents such as phenolics have been overlooked in all the studies on the foodstuffs such as cereals, pulses etc. But these constituents are found to play a greater role in the maintenance of the human body and therefore, have received a lot of attention of late. Studies carried out in this laboratory on vegetables and spices (Daniel 1989; Mangalan et al. 1989; Umadevi and Daniel 1990) have revealed that they contain a number of flavonoids and other phenolics, which are beneficial to the human body in many respects (Larson 1988; Harborne 1988). This communication presents data on the phenolic constituents of some of the common legumes.

All the seed materials were purchased from the markets in Baroda. These were (i) *Arachis hypogea* L. (Peanut / Groundnut, Mungphali), (ii) *Cajanus cajan* (L.) Millsp. (Cajan pea/Pigeonpea, Arhar/Tur), (iii) *Cicer arietinum* L. (Gram/ Chickpea, Chana), (iv) *Cicer kabulicum* Dixit (Kabul gram/ Kabuli chana), (v) *Cyamopsis tetragonoloba* Taub. (Cluster bean, Guar/Gawar), (vi) *Glycine max* (L.) Merrill. (Soybean, Bhat/Ramkurthi), (vii) *Lablab purpureus* L. (Indian bean, Sem), (viii) *Lens culinaris* Medic. (Lentil, Masur/Masar), (ix) *Pisum sativum* L. (Garden pea, Matar), (x) *Vicia faba* L. (Broad bean/ Windsor bean, Rajmah), (xi) *Vigna aconitifolia* (Jacq.) Marechal (Moth bean/Mat bean, Bhringga), (xii) *Vigna mungo* (L.) Hepper (Blackgram, Mung), (xiii) *Vigna radiata* (L.) Wilczek (Green gram, Mung) and (xiv) *Vigna unguiculata* (L.) Walp. (Cowpea/ Blackpea, Lobia/Chowli). The whole seeds were powdered using a laboratory blender and stored under refrigeration. The standard analytical procedures (Harborne 1984; Ibrahim and Towers 1960; Mabry et al. 1970; Markham 1982) involving interaction with diagnostic reagents and paper chromatographic separation of compounds and their UV/Visible spectroscopic studies including hypsochromic and bathochromic shifts with various reagents such as $AlCl_3$, $AlCl_3$/HCl, NaOMe, NaOAc and NaOAc/H_3BO_3 were followed for the identification of flavonoids and other phenolics. The identities of all the compounds were confirmed by co-chromatography (Paper chromatography and Thin layer chromatography) with authentic samples.

All the pulses screened were found to have various phenolics in them. Flavonoids,by far, were the most common compounds found to be present in 11 pulses. Among the flavonoids, flavonols were more common, seen in 10 seeds (Table 1). The flavonols identified were, kaempferol in *Arachis hypogea*, *Cyamopsis tetragonoloba*, *Lablab purpureus*, *Lens culinaris*, *Vicia faba* and *Vigna radiata* :3'-methoxy kaempferol in *Vigna aconitifolia*; 4'-methoxy kaempferol in *Lablab purpureus* and *Lens culinaris*; quercetin in *Cyamopsis tetragonoloba*, *Pisum sativum*, *Vicia faba*, *Vigna aconitifolia*, *V. radiata* and *V. unguiculata*; 3',4'-dimethoxy quercetin in *Cicer arietinum* and *Vigna unguiculata* and herbacetin in *Lens culinaris*. They were in traces in *Cicer kabulicum*, *Glycine max* and *Vigna mungo*. Anthocyanins such as cyanidin and pelargonidin were present in *Vicia faba* and delphinidin in *Arachis hypogea* (Table 1) Pro-anthocyanidins were present in *Lablab purpureus*, *Lens culinaris* and *Vigna unguiculata* (Table 1). Flavones as O-glycosides were found in 3 pulses. They were apigenin, 7-methoxy apigenin and luteolin in *Vigna mungo*, acacetin, 7-methoxy acacetin and apigenin *inv. radiata* and acacetin in *V. unguiculata*. Flavones as C-glycosides (Glycoflavones) were present in two plants only viz. 6-glucosyl acacetin and 8-glucosyl acacetin in *Vigna radiata* and isovitexin and vitexin in *V. mungo* (Table 2). Scopoletin, located in *Arachis hypogea*, was the only coumarin in the pulses screened. Phenolic acids were present in all the pulses (Table 3). Vanillic acid was omnipresent, while syringic, melilotic and *cis* and *trans*-ferulic acids were fairly common.

TABLE 1. DISTRIBUTION OF FLAVONOLS, ANTHOCYANINS AND PROANTHOCYANIDINS

Pulses	1	2	3	4	5	6	7	8	9	10
Arachis hypogea	+	-	-	-	-	-	-	+	-	-
Cajanus cajan	-	-	-	-	-	-	-	-	-	-
Cicer arietinum	-	-	-	-	+	-	-	-	-	-
Cicer kabulicum	-	-	-	-	-	-	-	-	-	-
Cyamopsis tetragonoloba	+	-	-	+	-	-	-	-	-	-
Glycine max	-	-	-	-	-	-	-	-	-	-
Lablab purpureus	+	-	+	-	-	-	-	-	-	+
Lens culinaris	+	-	+	-	-	+	-	-	-	+
Pisum sativum	-	-	-	+	-	-	-	-	-	-
Vicia faba	+	-	-	+	-	-	+	-	+	-
Vigna aconitifolia	-	+	-	+	-	-	-	-	-	-
Vigna mungo	-	-	-	-	-	-	-	-	-	-
Vigna radiata	+	-	-	+	-	-	-	-	-	-
Vigna unguiculata	-	-	+	+	+	-	-	-	-	+

Flavonols : 1-6, Anthocyanins : 7-9, Proanthocyanins : 10

1) Kaempferol, 2) 3-Methoxy kaempferol, 3) 4'-Methoxy kaempferol, 4) Quercetin, 5) 3',4'-Dimethoxy quercetin, 6) Herbacetin, 7) Cyanidin, 8) Delphinidin, 9) Pelargonidin 10) Proanthocyanidin

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TABLE 2. DISTRIBUTION OF FLAVONES AND GLYCOFLAVONES IN SOME PULSES

Pulses	1	2	3	4	5	6	7	8	9
Vigna mungo	-	-	+	+	+	-	-	+	+
Vigna radiata	+	+	+	-	-	+	+	-	-
Vigna unguiculata	+	-	-	-	-	-	-	-	-

Flavonols : 1-5, Glycoflavones : 6-9

1) Acacetin 2) 7-methoxy acacetin 3) Apigenin 4) 7-Methoxy apigenin 5) Luteolin 6) 6-Glucosyl acacetin 7) 8-Glucosyl acacetin 8) Isovitexin 9) Vitexin

TABLE 3. DISTRIBUTION OF PHENOLIC ACIDS IN PULSES

Pulses	1	2	3	4	5	6	7	8	9	10	11
Arachis hypogea	+	-	-	-	-	+	-	-	-	-	+
Cajanus cajan	-	-	-	+	-	-	-	-	-	+	+
Cicer arietinum	-	+	-	-	-	-	-	-	-	-	+
Cicer kabulicum	-	+	-	-	-	+	-	-	+	+	+
Cyamopsis tetragonoloba	-	-	-	-	-	+	-	-	-	+	+
Glycine max	-	-	-	-	-	+	-	-	-	+	+
Lablab purpureus	-	-	-	-	+	+	-	-	-	-	+
Lens culinaris	-	+	-	-	-	-	-	+	-	-	+
Pisum sativum	-	-	-	-	-	+	-	+	-	-	+
Vicia faba	-	+	-	-	-	+	+	-	+	-	+
Vigna aconitifolia	-	+	+	-	-	+	-	-	+	+	+
Vigna mungo	-	-	-	-	-	-	-	-	+	+	+
Vigna radiata	-	+	-	-	-	+	-	-	+	-	+
Vigna unguiculata	-	+	-	-	-	+	-	-	-	-	+

Coumarin : 1, Phenolic acids: 2-11

1) Scopoletin 2) *ρ*-Hydroxy benzoic acid 3) *o*- Coumaric acid 4) *ρ*-Coumaric acid 5) Chlorogenic acid 6) *cis trans*-Ferulic acid 7) Gallic acid 8) Gentisic acid 9) Melilotic acid 10) Syringic acid 11) Vanillic acid

Other phenolic acids such as *ρ*-hydroxy benzoic, *o*-coumaric, *ρ*-coumaric, chlorogenic, gallic and gentisic acids were found to be present in traces. It was observed that pulses that had less number of flavonoids had more number of phenolic acids.

The present study revealed the presence of phenolics in all the pulses studied. These findings are pertinent in the present context, as there is a need to search for special sources of antioxidants to be added to food. Several classes of flavonoids and phenolic acids showed antioxidant activity towards a variety of easily oxidizable compounds. Flavonols like kaempferol and quercetin are reported to have high antioxidant activities (Larson 1988). Anthocyanins protect tiny blood vessels from free radical damage and stimulate the formation of healthy connective tissue (Brown 1997). Since anthocyanins help in regenerating rhodopsin, a purple pigment needed for night vision and adaptation to light, a number of anthocyanin preparations from fruits such as blueberries and cranberries are flooding the market. It is to be remembered at this juncture that *Vicia faba* is a good source of anthocyanins, while *Lablab purpureus, Lens culinaris* and *Vigna unguiculata* contain proanthocyanidins, which on hydrolysis, yield anthocyanidins. Flavones like luteolin and 3',4'-dihydroxy flavone are potent inhibitors of the enzymes lipoxygenase and prostaglandin synthetase, which convert polyunsaturated fatty acids to oxygen containing derivatives (Larson 1988). Flavonoids with free hydroxyl groups at the 3',4'-positions (quercetin, luteolin) exert beneficial effects on the capillaries (De Eds 1968) by (1) chelating metals and thus sparing ascorbate from oxidation (2) prolonging epinephrine action by inhibiting O-methyl transferase and (3) stimulating the pituitary-adrenal axis. The flavonoids with multiple methoxy groups such as 3',4'-dimethoxy quercetin play an important role in circulatory system by reducing aggregation of erythrocytes (which occurs during illness or injury) by site-specific membrane surface effects and improve the microcirculation within the body (Srinivasan et al. 1971). Coumarin (scopoletin), which is available in peanut (*Arachis hypogea*) acts as diuretic, vasodilator and oestrogen (Wagner and Wolff 1977).

The identification of antioxidant flavonoids and other phenolics done in the present study will have to be followed by quantitative studies on these compounds in all the subspecies/varieties of these plants. The presence of these antioxidants provides added value to the pulses. This will lead to commercial utilisation of pulses as sources of these compounds and a better acceptance of them not only as sources of nutrients but also as a storehouse of antioxidants, which help in the maintenance of the body against oxidative damage caused by pollutants, sunlight etc. It is a known fact that Indian diets with good amounts of spices, vegetables and pulses are rich repositories of antioxidants.

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RESEARCH NOTE

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Oilgosaccharide Levels of Processed Redgram (*Cajanus cajan* L)

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The effects of pressure cooking and boiling, folowed by soaking of redgram seeds on the levels of raffinose family of sugars were investigated. Pressure cooking of redgram seeds for 15 min resulted in decrease of 50 % raffinose, 50 % stachyose and 50 % verbascose. Boiling followed by soaking was found to be an effective method for the removal of raffinose family of sugars, as the removal rates were 92% for raffinose, 80% for stachyose and 87% for verbascose.

Keywords : Redgram, Pressure cooking, Boiling, Oligosaccharides.

Redgram (*Cajanus cajan* L.) also called as pigeonpea is among the important grain legumes, which are grown and consumed in the tropics and semi-arid tropics of the world (Singh 1988). India accounts for about 90% of the world supply of redgram (Singh et al. 1990). It is also grown in some East African and South-east Asian countries. Most of the pigeonpea produced is consumed in the form of *dhal* curry in Northern India and *sambar* curry in Southern India. Green and immature seeds are also used as a vegetable in some States (Faris et al. 1987). Grain legumes including redgram are also rich source of proteins, vitamins especially of the B-complex and minerals such as calcium and iron (Meiner et al. 1976).

Redgram has been shown to contain many antinutritional factors and α-galactosides, which limit its wider use (Singh 1988). Most of the antinutritional factors including phytic acid, tannic acid, amylase and proteinase inhibitors can easily be removed by cooking and soaking methods (Singh 1988; Mulimani and Paramjyothi 1994). α-Galactosides include raffinose family or sugars such as raffinose, stachyose, and verbascose. These galactooligosaccharides constitute 53% of total soluble sugars. Of these antinutritional factors, the indigestible raffinose family of sugars (raffinose, stachyose, verbascose and ajugose) pass into the large intestine, where they are fermented anaerobically by the microbial flora, leading to flatus formation causing intestinal discomfort, nausea, abdominal rumbling and diarrhoea (Olson et al. 1981).

Simple processing like soaking, cooking and autoclaving have been shown to reduce the levels of raffinose family sugars in legumes (Singh 1988). Therefore, the present investigation was undertaken to study the effect of pressure cooking and boiling, followed by soaking for eliminating oligosaccharies from redgram flours.

Redgram seeds (whole seeds) variety 'local-1' and 'local-2' used in the present study were purchased from local market. All samples of redgram seeds were passed through a screen to remove debris and broken seeds and stored in polythene bags. Standard oligosaccharies were purchased from Sigma Chemical CO. St. Louis MO, USA. All reagents were of analytical grade.

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Determination of oligosaccharide content: Oligosaccharide concentration was determined in 1 g of powdered redgram flour according to the method of Tanaka et al (1975).

Total soluble sugars : Total soluble sugars in the concentrated sugar syrup were estimated by the phenol-sulphuric acid method described by Dubois et al (1956).

Estimation of reducing sugars : The amount of reducing sugars in the sugar syrup was determined by the method of Nelson (1944).

Effect of pressure cooking : Redgram seeds (100 g) were pressure cooked in a pressure cooker for 5, 10 and 15 min and dried in a hot-air oven at 50°C for 36 h and the dried seeds were milled to flour.

Effect of boiling followed by soaking : Seeds (100 g) were boiled in distilled water (1:10, bean to water ratio) for 10 min. The boiled seeds were soaked in the same water and removed at different time intervals of 4, 8, 12 and 16 h. The soaked seeds were dried in a hot-air oven at 50°C for 36 h, and the dried seeds were milled to flour.

Pressure cooking of redgram seeds resulted in decreases in the levels of raffinose family sugars (Table 1). Pressure cooking of redgram seeds for 15 min. resulted in mean decreases of 50 % raffinose, 50 % stachyose and 50% verbascose. Pressure cooking is an effective method for the removal of reducing sugars, sucrose and total soluble sugars (Table 1). The removal rates were 94 % for reducing sugars, 29% for sucrose and 42 % for total soluble sugars. Mulimani and Devindra (1998) reported decreases in the levels of raffinose family of sugars after cooking of redgram seeds for 60 min. Onigbinde and Akinyele (1983) reported that decrease in the levels of raffinose family sugars during cooking might be attributed to heat hydrolysis to disaccharides and monosaccnarides or to the formation of compound. Iyengar and Kulkarni (1976) have reported decreases in the levels of raffinose family of sugars after cooking of redgram and other legumes for 30 min.

Table 2 shows that boiling, followed by soaking for 16 h is very effective method for reducing the raffinose family sugars in redgram. Boiling followed by soaking led to significant decreases in the levels of raffinose family sugars compared to other methods described previously. Mean decreases of

TABLE 1. EFFECT OF PRESSURE COOKING ON THE LEVELS OF RAFFINOSE, STACHYOSE, VERBASCOSE, REDUCING, NON-REDUCING AND TOTAL SOLUBLE SUGARS OF REDGRAM (g/100g ON DRY BASIS)

		Pressure cooking, min			
	Variety	Raw	5	10	15
Raffinose	'Local 1	1.26	0.98	0.90	0.84
	'Local 2'	1.58	0.78	0.56	0.52
	Mean ± SD	1.42	0.88	0.73	0.68
		± 0.22	± 0.14	± 0.24	± 0.22
Stachyose	'Local 1	1.72	0.14	1.36	0.78
	'Local 2'	1.78	1.24	1.06	1.04
	Mean ± SD	1.75	0.69	1.21	0.91
		± 0.04	± 0.77	± 0.21	± 0.18
Verbascose	'Local 1	9.20	4.40	5.00	4.20
	'Local 2'	9.20	7.00	5.00	5.00
	Mean ± SD	9.20	5.70	5.00	4.60
		± 0.00	± 1.83	± 0.00	± 0.56
Reducing	'Local 1	0.86	0.08	0.07	0.08
sugars	'Local 2'	0.96	0.08	0.08	0.07
	Mean ± SD	0.91	0.08	0.07	0.07
		± 0.07	± 0.00	± 0.01	± 0.01
Sucrose	'Local 1	2.44	2.14	2.00	1.94
	'Local 2'	2.92	2.26	2.04	1.80
	Mean ± SD	2.62	2.20	2.02	1.87
		± 0.33	± 0.08	± 0.02	± 0.09
Total soluble	'Local 1	6.72	4.70	4.55	3.50
sugars	'Local 2'	5.64	3.80	3.70	3.60
	Mean ± SD	6.18	4.25	4.12	3.55
		± 0.76	± 0.63	± 0.60	± 0.07

Each value is a average of triplicate determination

92% raffinose, 80% stachyose and 87% verbascose were observed. Boiling followed by soaking was also an effective method for the removal of reducing sugars, sucrose and total soluble sugars (Table 2). Mean reductions of 80% reducing sugars, 91% sucrose and 30% total soluble sugars were observed. Olson et al (1981) developed a method of boiling, followed by soaking for removing sugars from dry beans. Whole beans were boiled for 3-4 min and then allowed to cool and stand in the same water for 16 h at room temperature. During this time, most of the oligosaccharides diffused into the water. By this method, over 90% of the family of sugars were removed from various beans.

The results of the present study have clearly shown that boiling followed by soaking for 16 h is an effective method for alleviation of flatulence inducing raffinose family sugars, thereby improving the nutritional quality of redgram. The method is simple and inexpensive for removal of oligosaccharides of redgram.

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TABLE 2. EFFECT OF BOILING, FOLLOWED BY SOAKING ON THE LEVELS OF RAFFINOSE, STACHYOSE, VERBASCOSE, REDUCING, NON-REDUCING AND TOTAL SOLUBLE SUGARS OF THE REDGRAM (g/100g ON DRY BASIS)

		Boiling, followed by soaking, h				
	Variety	Raw	4	8	12	16
Raffinose	'Local 1	1.26	0.88	0.30	0.16	0.12
	'Local 2'	1.58	0.16	0.24	0.18	0.10
	Mean ± SD	1.42	0.52	0.27	0.17	0.11
		± 0.22	± 0.50	± 0.04	± 0.01	± 0.01
Stachyose	'Local 1	1.72	1.22	1.12	0.36	0.34
	'Local 2'	1.78	1.18	0.48	0.38	0.36
	Mean ± SD	1.75	1.20	0.80	0.37	0.35
		± 0.04	± 0.02	± 0.45	± 0.01	± 0.01
Verbascose	'Local 1	9.20	3.00	3.40	1.60	1.20
	'Local 2'	9.20	2.80	2.40	1.40	1.20
	Mean ± SD	9.20	2.90	2.40	1.50	1.20
		± 0.00	± 0.14	± 0.00	± 0.14	± 0.00
Reducing	'Local 1	0.86	0.20	0.24	0.22	0.16
sugars	'Local 2'	0.96	0.22	0.28	0.30	0.20
	Mean ± SD	0.91	0.21	0.26	0.26	0.18
		± 0.07	± 0.01	± 0.02	± 0.05	± 0.02
Sucrose	'Local 1	2.44	1.64	0.78	0.38	0.20
	'Local 2'	2.92	0.90	0.86	0.66	0.30
	Mean ± SD	2.62	1.27	0.82	0.52	0.25
		± 0.33	± 0.52	± 0.05	± 0.19	± 0.07
Total	'Local 1	6.72	5.76	5.40	4.68	4.68
soluble	'Local 2'	5.64	4.32	4.24	4.12	4.00
sugars	Mean ± SD	6.18	5.04	4.82	4.40	4.34
		± 0.76	± 1.01	± 0.82	± 0.39	± 0.48

Each value is a average of triplicate determination

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RESEARCH NOTE

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Studies on the Chemical and Technological Aspects of Siahzira (*Carum gracile.* Lindl) Seed

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Proximate composition of *Carum gracile*. Lindl seeds and the physico-chemical characteristics of the volatile oils were determined. GC-MS analysis of the essential oil showed the presence of 31 constituents of which 22 compounds are reported for the first time. p-cymene (15.06%), γ-terpinene (28.95%), cuminaldehyde (16.59%), *p*-menth-1, 3-dien-7-al (10.89%), *p*-menth-1, 4-dien-7-al (9.51%), β-pinene (4.84%) and limonene (3.90%) were the major compounds. The yield of the volatile oil was 2.18% and that of the oleoresin was 8.66%, when extracted on a large scale. The oleoresin was found to keep well in cold (8-10°C) for 60 days even without the addition of antioxidants.

Keywords : Caraway, *Carum gracile,* Siahzira, Chemical composition, Essential oil, Oleoresin, Stability.

There are six species of the genus Carum found in India of which *Carum carvi* (caraway) is cultivated to a certain extent in colder regions of the country. The species *Carum gracile.* Lindl is indigenous to central plateau of Persia and Afghanistan. It is commonly found in the Ladakh region of Kashmir State at 2000-4000 m. The seeds are mostly imported and sold as a spice in India. The seeds are called 'Persian cumin', 'Siahzira' and 'Badakshani jeera'. The odour of the seed essential oil is sweeter than the normal cumin oil and little more reminiscent of caraway. As this is a very minor spice produce of India, the statistics on its area and production are not available (Wealth of India 1992; Pruthi 1976).

The literature survey on this seed spice shows meagre information. Krishna and Badhwar (1952) reported an yield of 2.5% essential oil from the seeds. The oil had an optical rotation of +7° and had 18% aldehydes. But, Agarwal et al (1979) reported an yield of 7.8% essential oil having an optical rotation of +46.65° and cuminaldehyde content of 27.57%. These variations may be due to differences in variety / cultivar, maturity, storage aspects etc.

As the information available on Siahzira is scanty, research work was carried out at this Institute on the chemical and technological aspects of C. gracile seeds. Large scale extraction of the essential oil and oleoresin, chemical composition of the essential oils and seeds were the important aspects of the work.

Preparation of samples : Seeds of Siahzira *(Carum gracile)* were procured from the markets of Mysore and Mumbai and the samples for analysis were prepared as per AOAC (1984) method. Seed samples (500 g each) were cleaned and powdered separately in a spice mill (Buhler Miag) to 500 μ size particles and the powders stored at 8-10°C in air-tight bottles. Also, 500 g seeds were roasted at 160°C for 15 min. powdered and stored in the same way as above. Seeds (500 g) were pre-conditioned with water and flaked in a triple roller.

Proximate composition : ASTA methods (1985) were used for determining the moisture and volatile oil contents and the volatile oils were made moisture-free by storing over anhydrous sodium sulphate at 8-10°C. The methods of ISI (1980 and 1982) were used to determine the ash content, water and solvent extracts, crude fibre, starch, nitrogen and protein contents. Total reducing sugars were determined by Lane and Eynon's method (David Pearson 1970). The oleoresin contents of the samples (500 g each) were determined by first removing the volatile oil by steam distillation and then extracting the spent powder with acetone (2.5 L) in a glass-column at room temperature. The acetone extract was desolventised on water bath and the resinous matter was mixed with its counterpart of the volatile oil to get the oleoresin content of the sample. The proximate composition data are given in Table 1.

Analysis of volatile oils : The physico-chemical properties of the volatile oils were determined by the methods given by Nigam (1966). The analytical values are given in Table 2. The GC-MS analysis of steam-distilled volatile oil (Mumbai) was carried out on Shimadzu - 17A chromatograph fitted with a QP-5000 quadrupole Mass-spectrometer. A fused silica SPB™-1 column 30 m x 0.33 mm (i.d), film thickness 0.25 μm coated with polydimethyl siloxane was used. The other parameters used for the analysis were : carrier gas helium with a flow rate

TABLE 1. PROXIMATE COMPOSITION OF SIAHZIRA SEEDS*

Characteristics, %	Mumbai	Mysore
Moisture	10.00	9.00
Total ash	7.87	9.30
Acid insoluble ash	1.16	1.21
Crude proteins	22.00	22.80
Crude fibre	15.00	17.00
Starch	16.50	17.39
Oleoresin	8.27	7.77
Total reducing sugars	13.78	11.80
NVEE	9.52	8.78
Acetone extract	12.36	12.77
Petroleum ether extract	8.38	7.91
Alcohol extract	16.27	15.28
Cold water extract	18.67	21.13
Cold alcohol extract	12.12	10.48

*Average of duplicate values

* *Corresponding Author*

TABLE 2. PHYSICO-CHEMICAL CHARACTERISTICS OF SIAHZIRA SEED VOLATILE OILS* (25-28°C)

	Mysore (Raw seed)			Mumbai (Raw seed)		Mumbai (Roasted)	
Characteristic	C.D.	S.D.	Flaked S.D.	C.D.	S.D.	C.D.	S.D.
Yield	2.95	2.50	2.70	3.10	2.50	3.10	2.45
Colour	L.Y.	L.Y.	L.Y.	L.Y.	L.Y.	L.Y.	L.Y.
Specific gravity	0.9385	0.9146	0.8812	0.9447	0.9202	0.9283	0.9170
Refractive index	1.4976	1.4932	1.4956	1.5016	1.4906	1.4946	1.4896
Optical rotation, 20°C	+6.64	+7.58	+6.90	+4.52	+5.65	+5.26	+5.72
Acid value	2.66	1.58	1.46	2.34	1.32	1.45	1.30
Evaporation residue, %	1.70	1.60	1.60	1.55	1.49	1.69	1.67
Solubility in 90% alcohol, vols	1:1	1:1	1:1	1:1	1:1	1:1	1:1
Aldehydes (as cuminal), % by HAH method	22.84	20.02	22.42	26.06	24.45	18.50	16.90

*Average of duplicate values
L.Y. = Light Yellow
C.D. = Clevenger Distilled
S.D. = Steam Distilled

of 1 ml/min; injection port temp. 250°C, detector port temp. 250°C and the oven temperature was kept at 50°C for 2 min, initially and then, it was increased to 250°C at the rate of 2°C per minute and maintained at 250°C for 5 min; ionisation voltage 70 ev. Retention indices of all the constituents were determined by Kovat's method using n-alkanes as standards. The constituents of the oil were identified by comparison of their Kovat's indices with those reported in literature and their identities confirmed by computer matching of their mass spectral fragmentation patterns with those of compounds in NIST-MS Library. The GC-MS analytical data are given in Table 3.

The major constituents of the distilled oils of Siahzira seeds were determined by using Shimadzu-15A gas chromatograph fitted with FID and SE-30 (10%) S.S. column (10ft x 1/8"). The GC analytical conditions were : detector port temp. 230°C; injection port temp. 220°C; chart speed 1 cm/min; carrier gas nitrogen at 35 ml/min and temp. prog. 75-5-200°C and 5 min hold at 220°C; sample dilution 1:10 with acetone and 1-2 µl injected at a time. Identification of the peaks was done by finding the retention times and peak enhancements with some reference chemicals. The major constituents of the volatile oils are shown in Table 4.

Storage stability of oleoresin : Samples (25 g each) of the oleoresin were separately treated with 0.02% BHA and TBHQ. The treated samples along with the controls were kept in cold (8–10°C) and at room temperature (25–30°C) for 60 days. Then, the samples were examined for their acid and peroxide values (Table 5) as per methods given by David Pearson (1970).

Large scale extractions : Steam distillation of the seeds was carried out by powdering 10 kg seeds to 0.5 - 1.0 mm dia. particles in an hammer mill and then subjecting the powder to steam distillation in a pilot-plant distillation unit for 2.5 h. The essential oil was collected over water in an oil-trap and the yield of the oil was 218 ml (2.18%). The oil was made moisture-free by storing over anhydrous sodium sulphate. A portion (1.82 kg) of the spent material obtained after the steam distillation was dried at 55°C and then extracted with acetone (10 L) in a glass

TABLE 3. GC-MS ANALYSIS OF SIAHZIRA SEED VOLATILE OIL (MUMBAI)*

Peak No	RT	Compound	K I	% Peak area
1	5.15	α - Pinene*	923	2 74
2.	6.46	β - Pinene	962	4 84
3	7.24	β - Myrcene	981	1 62
4	8.27	*p*-Cymene	1010	15 06
5	9.03	Limonene*	1022	3 90
6	10.43	γ - Terpinene	1052	28.95
7	11.70	Terpinolene*	1074	0.27
8	11.86	Menth-2-en-l-ol*	1077	0.09
9	12.26	Linalool	1084	0.12
10	12.57	*p*-1,3,8-Menthtriene*	1089	Tr
11	13.46	*Cis*-Limonene oxide*	1103	0 05
12	13.70	*Cis*-Pinene hydrate*	1108	0.08
13	13.91	1-Terpineol*	1112	0.04
14	14.06	*Trans*-Limonene oxide*	1115	0 05
15	15.29	*Cis*-Verbenol*	1137	0.54
16	16.04	4-Terpineol*	1149	0.73
17	16.54	*Trans*-Verbenol*	1158	1 95
18	16.84	α - Terpineol	1162	Tr
19	19.84	Cuminaldehyde	1220	16 59
20	22.87	*p*-Menth-1, 3-dien-7-al*	1257	10 89
21	23.56	*p*-Menth-1, 4-dien-7-al*	1273	9 51
22	24.34	Thymol	1278	Tr
23	24.44	Dehydro elsholtzia ketone*	1280	Tr
24	24.73	Carvacrol	1284	0.04
25	31.08	*Cis* - β - Farnesene*	1382	0 07
26	34.67	Germacrene-D*	1440	0.11
27	36.15	α - Zingiberene*	1464	0 09
28	37.00	*Trans*-β- Farnesene*	1477	0 12
29	37 66	*Trans*-α - Farnesene*	1487	0 11
30	39 75	*Cis*-Nerolidol*	1522	0 04
31	46 47	*Epi*-α- Bisabolol*	1636	0 26

* Steam distilled oil
* Reported for the first time
RT : Retention Time
KI Kovat's Index values on SPB-1 Column

TABLE 4. CONSTITUENTS OF SIAHZIRA SEED VOLATILE OILS (%) BY GC ANALYSIS

Type of oil	α-Pinene	β-Pinene +β-Mycene	p-Cymene + Limonene	γ-Terpinene	Terpin-olene	Trans-Verbenol	Cuminal-dehyde	p-Menth-1, 3/4-dien-7-al	Germa-crene-D	α-Zinge-berene	α-Farne-sene + β-Farnesene
Mysore C.D.	1.54	3.64	19.56	28.03	0.76	0.86	24.49	14.08	1.61	1.32	1.82
Mysore S.D.	2.50	5.08	21.01	31.96	0.81	1.14	18.91	11.07	1.54	1.23	1.82
Mysore Flaked	1.81	4.09	18.87	30.49	0.79	1.24	21.07	13.67	1.89	1.94	2.16
Mumbai C.D.	0.86	2.41	18.35	25.03	0.36	1.81	27.72	21.76	0.08	0.06	0.07
Mumbai S.D.	2.00	4.35	19.66	37.36	0.50	1.55	17.69	15.28	0.02	0.16	0.18
Mumbai Roasted C.D.	1.89	3.98	18.66	33.14	0.49	0.75	17.61	20.22	0.02	0.18	0.48
Mumbai Roasted S.D.	2.22	4.59	19.52	39.70	0.56	1.54	14.60	16.14	0.02	0.11	0.17

C.D. = Clevenger Distilled
S.D. = Steam Distilled

column. The yield of the resinous matter was 118 g (6.48%). Then, the corresponding quantity (40 g) of the volatile oil was added to the resinous matter and thus, the yield of the oleresin was 8.66%.

Proximate composition of seeds : The analytical values (Table 1) for moisture, volatile oil, ash, protein, fibre, starch, total reducing sugars and oleoresin showed that there was not much difference among Mysore and Mumbai samples in proximate composition. These analytical values may help in framing standards/specifications for Siahzira and its products.

Analysis of volatile oils : The yield of the volatile oils and their physico-chemical properties are given in Table 2. The yield of the volatile oil was not improved either by flaking or by roasting the seeds. All the oils were soluble in 1 volume of 90% alcohol. The optical rotation ranged from +66° to +80° in case of *Carum carvi,* while it ranged from +4.50 to +6.90 in case of *Carum gracile.* This may be due to the presence of carvone (50-75%) in *C. carvi* and aldehydes (16.50 – 26.6%) in case of *C. gracile.*

The GC-MS analysis of the steam distilled volatile oil has shown the presence of 31 constituents of which twenty two compounds are being reported for the first time. Mono-terpenoids constituted 97-98% of the oil. Remaining 1-2% of the oil consists of sesquiterpenes. Terpene hydrocarbons viz., p-cymene, γ-terpinene, β-pinene and limonene were the major compounds, accounting for 57-60% of the oil. Aldehydes, which contribute to the characteristic flavour of this seed spice accounted for 37-39% of the constituents. Cuminaldehyde, *p*-menth-1, 3-dien-7-al, and *p*-menth-1, 4-dien-7-al were the three major aldehydes present in this oil. Alcohols were the minor compounds of the oil.

The GC analysis of the volatile oils has shown the following major constituents (%) : p-cymene + limonene, γ-terpinene, cuminaldehyde and *p*-Menth-1, 3-dien-7-al + *p*-Menth-1, 4 dien-7-al. The contents of aldehydes were more in the oils obtained by C.D. (Table 4). No significant differences were found in the chemical compositions of flaked and roasted seeds (Table 4). The volatile oil of *C. gracile* differs from that of *C. carvi* which is rich in carvone and limonene (Lawrence 1980).

TABLE 5. STORAGE STABILITY OF SIAHZIRA SEED OLEORESIN*

Treatment	Acid value	Peroxide value mg/kg
At 8–10°C		
(a) Control	7.42	10.29
(b) With BHA	6.65	9.42
(c) With TBHQ	7.14	9.36
At 25–30°C		
(a) Control	12.07	30.18
(b) With BHA	10.93	26.45
(c) With TBHQ	11.02	28.53

* Average of duplicate values
Initial acid value : 5.15
Initial peroxide value : 7.86 meq/kg

Oleoresin stability : The results of the stability studies showed that the oleoresin could be preserved well even without the addition of antioxidants like BHA or TBHQ, when kept in cold temperature (8–10°C) and the antioxidants failed to protect the quality of the oleoresin, when it was stored at high temperature (25-30°C) for 60 days (Table 5).

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RESEARCH NOTE

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Biochemical and Microbiological Changes During Storage of Smoked *Puntius sophore* Obtained from Market

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Storage life of smoked *Puntius sophore* stored in split bamboo box at room temperature was found to be 120 days (April to August). Total plate counts of bacteria (TPC), fungi (TFC), *Staphylococcus aureus,* faecal *Streptococci* and most probable number (MPN) of coliform gradually decreased with decrease in moisture content of the fish. During rainy season, TPC and TFC increased correspondingly with increases of humidity in the environment and moisture content of the fish. Most probable number of coliform, *Staphylococcu aureus* and faecal *Streptococci* were not detected in samples stored for 39. 60 and 75 days. *E. coli* and *Salmonella* were not detected in any of the samples analysed. *Aspergillus niger* was dominant in April, May and June, whereas *A. flavipes* and *A. nidulans* were dominent in July and August. Growth of fungi was found to be rapid in the rainy season.

Keywords : Smoked *Puntius sophore*, Biochemical and micro biological changes, Storage life, Microflora.

Small sized trashfishes are not readily acceptable to consumers because of low meat yield, bony nature and poor taste. However, smoked freshwater carp minnow, *Puntius sophore* is widely consumed in Manipur. The fish thus processed is consumed after frying or roasting or as an ingredient in vegetable curry preparations in order to add flavour and taste (Singh et al. 1990). The fish is smoked by villagers in traditional Manipuri style and sold in the market. These are stored in small boxes made of thin split bamboo (called 'Ngarubak' in Manipuri). As microganisms usually grow on nutrient-rich organic substances, there is a possibility of incidence of such organisms in fish samples during storage. Some of such organisms cause health hazards to consumers. This communication deals with changes in the microflora of smoked *Puntius sophore* during storage in split bamboo boxes. Attempts have also been made to correlate the data with the moisture content of the fish and also with environmental parameters.

Smoked *Puntius sophore* samples were collected from 6 different fish sellers of Imphal market in the month of April, 1997. They were packed separately and then brought to the laboratory. They were then stored separately in the bamboo boxes.

Sensory evaluation : Colour, texture and odour of the fish were judged by a panel of 7 judges. Colour was recorded based on visual observation, texture by applying pressure by fingertips and odour by smelling on a 3 - point Hedonic scale as: good, medium and poor. Organoleptic scores were assessed on a 10-point Hedonic scale, 10 being very good, 3 being unacceptable and 1 being bad taking into consideration the changes in odour, texture and flavour of the fish. Moisture content of the fish was determined by air oven method (AOAC 1975). Total volatile base nitrogen (TVBN) was determined as per the method of Morris (1959).

Environmental parameters : Temperature and humidity were recorded using a maximum and minimum thermometer and a hygometer, respectively during the entire period of study.

Microbiological quality : Enumeration of TPC and TFC and most probable number (MPN) of coliform and detection of pathogenic bacteria, viz., *Salmonella, Escherichia coli, Staphylococcus aureus* and faecal *Streptococci* were done as per the procedure of APHA (1976). TPC was enumerated on plate count agar, TFC on acidified potato dextrose agar (PDA), most probable number (MPN) of colifroms on brilliant green lactose bile (BGLB) broth, *E coli* on eosine methylene blue (EMB) agar after enrichment in BGLB broth, *Salmonella* on brilliant green agar (BGA) after enrichment in selenite cystine broth (SCB) *Staphylococcus aureus* on Baird Parker agar (BPA) following treatment in egg yolk tellurite and faecal *Streptococci* on KF Streptococcal agar containing triphenyl tetrazolium chloride. The suspected pathogenic bacterial colonies were further tested by the methods of APHA (1976) and Kiss (1984). Fungal colonies on PDA were picked, stained with cotton blue in lactophenol and identified, based on the methods of Gilman (1957) and Ellis (1971, 1976).

Data on TVBN, pH and sensory evaluation of smoked *Puntius sophore* are shown in Table 1. pH values showed that the products were acidic. It may be due to the effect of phenolic/acidic constituents deposited on the fish muscle during smoking. TVBN value were in the range of

TABLE 1. pH, TVBN AND SENSORY ATTRIBUTES OF SMOKED *PUNTIUS SOPHORE*

Sample	pH	TVBN, mg %	Organoleptic properties		
			Texture	Colour	Smoked odour
P_1	6.70	40.00	Brittle	Yellowish black	Medium
P_2	6.41	60.00	Brittle	Golden yellow	Medium
P_3	6.50	60.00	Brittle	Golden yellow	Medium
P_4	6.81	45.00	Crisp	Yellowish black	Medium
P_5	6.51	40.00	Crisp	Blackish yellow	Medium
P_6	6.42	60.00	Brittle	Golden yellow	Medium

The values and observations are mean of 6 samples each of smoked *P.sophore*

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TABLE 2. MOISTURE, ENVIORNMENTAL PARAMETERS, VISIBLE FUNGAL COLONIES, BACTERIAL AND FUNGAL POPULATION CHANGES DURING STORAGE OF SMOKED *P. SOPHORE*

Days	Moisture, %	Temp,°C	Humidity,%	Visible fungal colony	TPC, cfu g^{-1}	TFC, cfu g^{-1}	*Staphyloc, aureus*, cfu g^{-1}	*Faecal Streptoco*, cfu g^{-1}	MPN of *coliform* cfu g^{-1}	Organo-leptic score
0	9.35 ± 2.20	19.95	73.5	Nil	2.60×10^5	8.06×10^5	3.01×10^3	5.41×10^2	1.00×10^2	9
15	7.20 ± 1.00	22.32 ± 6.90	71.60 ± 6.90	Nil	3.90×10^4	4.61×10^4	6.00×10^2	2.10×10^2	1.00×10^2	8
30	8.10 ± 1.50	24.46 ± 1.74	66.32 ± 13.3	Nil	2.10×10^4	3.00×10^3	2.07×10^2	7.00×10^2	Nil	8
45	10.80 ± 1.43	25.30 ± 1.50	76.50 ± 10.5	Nil	2.00×10^4	7.70×10^3	1.81×10	7.10×10	Nil	7
60	11.60 ± 1.20	26.10 ± 1.20	85.70 ± 6.90	Nil	6.80×10^3	4.00×10^2	Nil	2.00×10	Nil	7
75	10.26 ± 0.81	26.12 ± 1.22	86.30 ± 4.85	Nil	2.93×10^3	1.00×10^2	Nil	Nil	Nil	6
90	12.38 ± 1.34	25.85 ± 1.30	86.60 ± 5.40	+	1.00×10^3	5.61×10^3	Nil	Nil	Nil	5
105	11.31 ± 0.55	26.49 ± 0.75	82.07 ± 5.20	+	3.46×10^3	2.07×10^4	Nil	Nil	Nil	4
120	10.12 ± 1.40	25.85 ± 0.68	78.09 ± 4.78	++	7.04×10^4	4.11×10^4	Nil	Nil	Nil	4

Note - *E. coli* and *Salmonella* were not detected in all the samples
+ = a few scattered colonies, ++ = many isolated colonies
Results are Mean ± SD of 6 samples

TABLE 3. FUNGI ISOLATED DURING STORAGE OF SMOKED *PUNTIUS SOPHORE*

Days	Fungi
0	* *Aspergillus niger, Penicillium* spp, Sterile mycellium
15	* *A. niger, Penicillium rubrum, P. funiculosum*
30	* *A niger,* White sterile *mycellia, Penicillium* spp., *A. candidus*
45	* *A niger, Penicillium* spp., *A. candidus, Trichoderma longibranchiatum*
60	* *A. niger, A. candidus, Penicillium* sps.
75	* *A. niger, Penicillium* spp., *A. flavipes, A. repens*
90	* *A. flavipes, *A. nidulans, Penicillium* spp., *A. repens*
105	* *A. flavipes, A nidulans, Penicillium* spp., *A. repens*
120	* *A. flavipes, A nidulans, Penicillium funiculosum, P. rubrum*

*- Dominant fungi

40.0-60.0mg %. This high value of TVBN after smoking might be probably due to the subsequent microbiological and biochemical change in the fish muscle during smoking. Joseph et al (1987) also observed TVBN value of 70.0 mg % in smoked barracuda. But this is not reflected in the organoleptic qualities of smoked fish (Joseph et al. 1987). Texture of the fish was crisp to brittle and the colour was from yellowish black to golden yellow. All the samples had a medium smoky odour.

A relationship between moisture, meterogical data and microflora of the fish during storage is shown in Table 2. A moisture content of 9.35 % was observed on the initial day. It gradually decreased to 8.1 % after 30 days, of storage. Then, the value increased gradually to 12.38% on the 90th day. Further storage resulted in decrease of moisture content. In most of the present study period, humidity was above 70%. Moisture also plays an important role in the spoilage of fish and lowering of moisture retards the spoilage of fish (Stansby 1963). Fungal colony was visible after 90 days of storage. Higher rainfall was also recorded on 90 days of storage. Rapid growth of visible fungi colonies were observed in rainy season. *Staphylococcus aureus,* faecal *Streptococci* and coliforms were not detected on 60 days of storage, 75 days of storage and 30 days of storage samples. *E.coli* and *Salmonella* were not detected in all the smoked fish samples analysed.

Table 3. shows fungi isolated during storage of smoked *Puntius sophore.* In the present finding, most of the isolated fungi were *Aspergilli* and *Penicillia.* According to Frazier and Westhoff (1978), the most common and obvious causes of spoilage were *Penicillia* and *Aspergilli.* In the present analysis, xerophilic fungi, *A. niger, A. candidus* were observed. In April, May and June, the dominant type was *A.niger.*

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RESEARCH NOTE

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Variation and Relationship Among Cooking Quality Attributes Across the Environments in 'Kabuli' Chickpea (*Cicer arietinum* L.)

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Fiftyfive 'Kabuli' chickpea genotypes were studied for physical characters to assess their role in cooking quality across the four environments. Seed mass, seed volume, swelling capacity and hydration capacity were important quality attributes. These characters were also mutually correlated. The genotypes 'ICARDA-112', 'HK 92-103', 'ICARDA-09101', 'GNG 827' and 'HK 92-117' were found to be promising genotypes to throw desirable segregants for cooking quality in breeding programmes.

Keywords : 'Kabuli' chickpea, Cooking quality, Segregants, Environment.

'Kabuli' chickpeas have a good cooking quality and are nutritionally superior than 'Desi' chickpeas. The biological value of 'Kabuli' types is considerably higher than 'Desi' types and the former types contain more utilizable proteins, Polyphenolic compounds in 'Desi' are more than twice those of 'Kabuli' (Singh and Jambunathan 1981; Singh et al. 1991). For cooking quality, seed mass, seed volume, hydration capacity and swelling capacity have been observed to be mutually correlated. However, there are contradictory reports with regard to association of these attributes with cooking time (Williams et al. 1983; Badshah et al. 1987; Waldia et al. 1996). There are a few studies in literature on cooking quality of 'Kabuli' chickpea based on different growing conditions. The performances of characters are found to be influenced by differential response to growing seasons and conditions (Williams and Singh 1987; Singh et al. 1990; Aparna et al. 2000). Therefore, the present study pertains to variation and relationship among cooking quality traits under four growing conditions, in 55 'Kabuli' chickpea genotypes.

Fifty five 'Kabuli' chickpea genotypes were obtained from different centres of national and international centres, growing chickpea. These were grown in four different environmental conditions generated over two years (1993/94 and 1994/95 post-rainy seasons) and plated at two sowing dates in each season at the farms of pulses research area, CCS Haryana Agricultural University, Hisar, India. (Table 1).

The genotypes were accomodated in 4 m long single row plots, spaced 30 cm apart; distance among plants within row being 15 cm, following a randomised block design with three replications under each environment. Normal recommended agronomical practices were followed to raise the crop. The seeds of each genotype per replication were harvested from each environment during both the years for laboratory tests. Laboratory tests for each character were carried out in triplicates and the mean values were used for statistical analysis.

The physical characters, namely, seed mass (50 seed weight) and seed volume were determined by the method employed by Williams et al (1983). To determine hydration capacity and swelling capacity, 50 seeds of known weight were incubated in 100 ml distilled water overnight at room temperature (22°C) by employing the method of Williams et al (1983). Excess water was drained off and the surface water was removed with absorbant paper, before recording the volume and weight of swollen seeds. Hydration and swelling indices were determined as; hydration index = (hydration capacity/seed)/original seed weight (g); whereas, swelling index = (swelling capacity per seed)/seed volume (ml).

TABLE 1. DETAILS OF THE FOUR ENVIRONMENTS FOR TESTING THE GENOTYPES

Environment (E)	Year	Date of sowing
E1	1993	28.11.1993
E2	1993	21.12.1993
E3	1994	25.11.1994
E4	1994	17.12.1994

To determine the cooking time (minutes), a small container of 25 ml capacity was filled with seeds of a known weight and placed in a 250 ml conical flask. The samples were strirred at 2 min. interval, to facilitate uniform heating at all the times. The degree of cooking was judged visually by the seed pressing between fingers till softness.

Correlation at phenotypic and genotypic levels were calculated from variance and convariance according to Johnson et al (1955) in all the 55 genotypes under different environments.

Sowing dates caused significant changes in the environments for all the genotypes in respect of all characters (Table 2). The artificially induced environments extended the range of environment index values, indicating the utility of these environments.

The results given in Table 3 indicate considerable variations in all the characters. The seed mass (50 seed weight) varied from 7.66 ('BG-267') to 16.99 ('ICARDA-112'), whereas seed volume (ml/seed) varied from 7.68 ('KGK-9') to 12.78 ('GNG-827'). Variations for hydration capacity (g/seed) were 0.18 ('BG-267') to 0.32 ('HK-92-110' and 'HK 92-103'); for hydration index (g/seed) from 0.94 ('HK 92-96') to 1.16

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TABLE 2. ENVIRONMENT-WISE ANALYSIS OF VARIANCE FOR PHYSICAL CHARACTERS IN 'KABULI' CHICKPEA (*CICER ARIETINUM* L.)

Character	Source	df	Mean squares E1	E2	E3	E4
Seed mass	Replications	2	0.351	1.217	1.712	0.953
	Genotypes	54	22.438**	16.427**	14.247**	12.623*
	Error	108	0.339	0.831	0.616	0.577
Seed volume	Replications	2	0.575	0.409	0.597	0.128
	Genotypes	54	12.624**	7.500**	7.907**	8.816**
	Error	108	0.246	0.133	0.395	0.118
Hydration capacity	Replications	2	0.001	0.000	0.0001	0.0001
	Genotypes	54	0.007**	0.0050**	0.0054**	0.0040**
	Error	108	0.001	0.0001	0.0001	0.0001
Hydration index	Replications	2	0.001	0.010**	0.0006	0.001
	Genotypes	54	0.0080*	0.031**	0.0072**	0.021**
	Error	108	0.0004	0.001	0.005	0.001
Swelling capacity	Replications	2	0.000	0.0001	0.0001	0.0001
	Genotypes	54	0.006**	0.0040**	0.0055**	0.0040*
	Error	108	0.0001	0.0001	0.0001	0.0001
Swelling index	Replications	2	0.002	0.002	0.001	0.003
	Genotypes	54	0.024**	0.049**	0.039**	0.040*
	Error	108	0.002	0.002	0.002	0.001
Cooking time	Replications	2	1.57	2.37	1.352	1.509
	Genotypes	54	272.959**	262.356**	266.003**	266.442
	Error	108	0.792	1.049	1.092	0.460

E1 = Environment 1; E2 = Environment 2; E3 = Environment 3; E4 = Environment 4

* and ** significant at 5 and 1% levels, respectively

TABLE 3. MEAN, RANGE AND PROMISING GENOTYPES FOR PHYSICAL CHARACTERS IN 55 'KABULI' GENOTYPES ACROSS THE FOUR ENVIRONMENTS

Character	Range	Mean	C V,%	Promising genotypes
Seed mass, g (50 seed weight)	7.66 -16.99	13.52 ± 0.85	6.28	'ICARDA'-112' (16.99), 'HK 92-103' (16.98), 'ICARDA-09101' (16.32), 'GNG-827' (16.11) 'HK 92-117' (15.84)
Seed volume, m1/speed	7.68 -12.78	10.72 ± 0.82	7.66	'HK 92-103' (12.91). 'GNG-827' (12.78), 'ICARDA-09101' (12.72) 'HK 92-110' (12.50). 'HK 92-87' (12.48)
Hydration capacity, g/seed	0.18 -0.32	0.26 ± 0.04	15.38	'HK 92-110' (0.32), 'HK 92-102' (0.32) 'ICARDA-0901' (0.31), 'GNG-827' (0.31, 'HK 92-97' (0.31)
Hydration index, g/seed	0.94 -1.16	1.02 ± 0.16	15.68	'HK 92-114' (1.16), 'L550' (1.15) 'HK 89-134' (1.10). 'KGK-9' (1.12)
Swelling capacity, m1/seed	0.19 -0.34	0.26 ± 0.03	11.53	'GNG-827' (0.34), 'HK 92-97' (0.33) 'HK 92-103' (0.32), 'ICARDA-09101' (0.31), 'HK 92-110' (0.31)
Swelling index, m1/seed	1.08-1.34	1.2 ± 0.1	8.33	'HK 92-114' (1.34), 'HK 89-96' (1.33) 'HK 92-85' (1.32), 'HK 92-112' (1.31) 'HK 89-129' (1.31)
Cooking time, min	45.50 -108.42	57.28 ± 2.31	4.03	'HK 92-201' (45.50). 'HK 92-111' (45.58), 'HK 91-111' (47.00) 'ICARDA-09101' (48.17) 'HK 89-129' (49.50)

Mean ± Standard error ; CV = Coefficient of variation

('HK 92-114'); whereas for swelling capacity (ml/seed) it ranged from 0.19 ('KGK-9'). Variations in cooking time were from 45.50 ('HK 92-201') to 108.42 ('ICARDA-09103'). The genotypes showed wide variations for different traits across the four environments and could be used in quality improvement programme.

The genotypes, viz., 'ICARDA-112', 'HK 93-103', 'ICARDA-09101', 'GNG-827' and 'HK 92-117' were found to be promising, as they possessed higher seed mass, seed volume, hydration capacity and swelling capacity. These genotypes also took less time for cooking. The higher seeded genotypes reduce seed coat (%) and hull thickness (Waldia et al 1995, 1996). Low seed coat (%) and hull thickness are very desirable characteristics for cooking quality (Williams and Singh 1987). A low seed coat raises the theoretical maximum extractions of endosperm and increases the energy available to monogastric animals (Knights 1989). The genotype 'ICARDA-09101' was the most promising, as it had higher seed mass, seed volume, hydration capacity and swelling capacity; besides low cooking time. Such a genotype should be used extensively in crossing programmes to obtain desirable segregants for cooking quality.

The associations in 55 'Kabuli's genotypes were analysed under four environments. The results are given in Table 4.

The phenotypic and genotypic correlation coefficients among physical characters revealed that the genotypic correlation coefficients were higher than their corresponding phenotypic correlation coefficients. Seed mass under all environments had positive correlations with seed volume, swelling capacity and hydration capacity. The positive and significant correlation of seed mass and its associated attributes viz., seed volume, hydration capacity and swelling capacity were not noticed with cooking time. Environment 1 was an exception to this, where the swelling capacity had positive association with cooking time. These findings are in agreement with those reported by Waldia et al (1996). However, for cooking time, Williams et al (1983) and Badshah et al (1987) reported positive and significant correlations of seed mass with cooking time. Singh et al (1991) reported that larger seeds did not necessarily take longer time for cooking. The relation of cooking time with seed mass and its associated attributes did not persist to the same extent under all conditions. This may be attributed to difference in the physical hardness of the sample, caused by different responses to the growing environments (Williams and Singh 1987).

Quite often, cooking quality has been considered a function of cooking time. Singh (1999) has emphasized the importance of seed size and cooking time, besides other characters, which influence cooking quality. Cooking quality, depends upon growing conditions of legumes (Aparna et al. 1990). Singh et al (1990) reported that growing season had a marked effect on seed weight and cooking time in 'Kabuli' chickpea. Therefore, for selection of superior gentotypes for cooking quality, environmental factors should be given due consideration. The present study has indicated that the genotypes; 'ICARDA-112', 'HK 92-103', 'ICARDA-09101', 'GNG-827' and 'HK 92-117', which possessed high seed mass and

TABLE 4. ENVIRONMENT-WISE GENOTYPIC (G) AND PHYNOTYPIC (P) CORRELATION COEFFICIENTS AMONG PHYSICAL CHARACTERS IN 'KABULI' CHICKPEA (*CICER ARIETINUM* L.)

Character		1	2	3	4	5	6	7
Seed mass	E^1 G		0.335	0.349	-0.042	0.337	-0.018	-0.135
	P		0.325*	0.342	0.042	0.325*	-0.008	-0.129
	E^2 G		0.303	0.300	-0.103	0.274	-0.012	0.114
	P		0.291*	0.279	0.099	0.257*	-0.007	0.108
	E^3 G		0.279	0.308	-0.144	0.287	0.353	0.003
	P		0.265*	0.299*	0.131	0.272*	0.323*	0.001
	E^4 G		0.265	0.262	-0.014	0.273	-0.103	0.097
	P		0.252*	0.256	-0.012	0.163*	-0.099	0.093
Seed volume	E^1 G			0.091	0.084	0.247	-0.227	0.265
	P			0.077	0.055	0.195	-0.189	0.215
	E^2 G			-0.243	-0.154	0.174	-0.081	-0.051
	P			0.216	-0.136	0.153	-0.066	-0.047
	E^3 G			0.272	0.083	0.354	0.222	-0.066
	P			0.267*	0.057	0.304*	0.169	-0.057
	E^4 G			0.042	-0.073	0.031	0.224	0.019
	P			0.031	-0.078	0.017	0.203	0.018
Hydration capacity	E^1 G				0.059	-0.119	0.019	0.033
	P				0.049	0.111	0.002	0.029
	E^2 G				-0.107	0.039	-0.108	-0.255
	P				-0.098	0.029	-0.101	-0.227
	E^3 G				-0.115	0.063	-0.242	0.088
	P				-0.103	-0.054	-0.225	0.088
	E^4 G				-0.225	0.22	0.134	0.015
	P				-0.214	0.213	0.133	0.014
Hydration index	E^1 G					-0.104	0.176	-0.108
	P					-0.101	0.175	-0.104
	E^2 G					-0.041	-0.227	0.192
	P					-0.042	-0.207	0.183
	E^3 G					0.099	-0.236	-0.007
	P					0.099	-0.222	-0.008
	E^4 G					0.009	-0.041	0.011
	P					0.019	-0.037	0.012
Swelling capacity	E^1 G						-0.011	0.317
	P						0.007	0.284*
	E^2 G						0.042	0.124
	P						-0.107	0.149
	E^3 G						0.326	0.045
	P						0.280*	0.041
	E^4 G						0.147	0.041
	P						0.144	0.127
Swelling index	E^1 G							-0.186
	P							-0.176
	E^2 G							-0.175
	P							-0.163
	E^3 G							-0.124
	P							-0.119
	E^4 G							0.018
	P							0.019

*Significant at 5 percent level of significance

low cooking time across the four environments should be utilized in breeding programmes aimed at improving the cooking quality of 'Kabuli' chickpeas.

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RESEARCH NOTE

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Preservation of Mutton as Ready-to-Eat Curry by Hurdle Technology

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Conditions for the preparation of mutton curry suitable to Indian palate were standardized. The mutton curry thus prepared was preserved by hurdle technology, employing various hurdles like water activity (a_w), pH, preservative (pres.), high temperature (F), low temperature (t) and oxido-redox potential (Eh) to obtain convenience intermediate moisture product, which after a few minutes of heating in boiling water could be served in the curry form. The product having 37.68% moisture, 33.15% fat and 5.58 pH was stable for more than four months at ambient temperature of 27± 2°C and more than six months at refrigerated temperature of 3 ±2°C. Chemical quality indices like free fatty acids (FFA), peroxide value (PV) and thiobarbituric acid value (TBA), texture (Warner-Bratzler shear value) as well as microbial and sensory qualities were evaluated and statistically analysed. Changes of these quality parameters during storage were monitored and tabulated. The product had a longer storage life at refrigeration than at ambient temperature and in paper-foil-polyethylene pouches than in polypropylene pouches.

Keywords : Mutton curry, Shelf stable, Ready-to-Eat, Hurdle technology, Storage, Packaging, Temperature.

India being a major livestock producer, has a global share of 4.34% and 17.15% of sheep and goat population, respectively (FAO 1995 ; 1997). In 1997, India generated 4.706 million tonnes of sheep and goat meat with an average annual growth rate of 15.85% since 1991. This production amounted to 21.49% of the world mutton production and 16.09% of India's meat production (FAO 1998). Surplus over domestic consumption of mutton was exported, fetching foreign exchange worth Rs. 71.04 crore in 1996-97. There is a great opportunity for processed meat products to be marketed in India as well as in importing countries (Chatterjee 1999).

Hurdle technology ensures optimal combination of hurdles (Berwal 1994), which act additively or synergistically (Brimelow 1985; Leistner 1994; Grijspaardt 1994). In preservation of meat and meat products of several continents, this concept has been applied by various researchers (Hechelmann et al. 1991; Leistner 1985; Manish and Berwal 1996; Karthikeyan 1997; Wang and Leistner 1993; Modi et al. 1999, Himanish and Sumithra 1998). In meat products, a_w can be reduced by dehydration, cooking or addition of humectant. Cooking helps to reduce the microbial load of the product. Several acidulants are used to reduce pH of the meat. In addition to its acidulant property, citric acid enhances the inhibitory property of potassium sorbate against *Clostridium botulimum* in ham and bacon (Webster and Cooke 1984). As an antimold agent, potassium sorbate added to beef reduces contamination remarkably (Zaemora and Zartizky 1987) and its dipping in 5% solution prolongs the shelf life of beef. Various spices and condiments are known for their antimicrobial and antioxidant properties (Rajalakshmi and Narasimhan 1996; Kikuzaki and Nakatani 1993; Chipault et al. 1955). Meat curry in India is a traditional meat product, which is eaten afresh. According to Singh et al (1998), meat curry is one of the most relished meat products of the eastern part of the country. These curried preparations are perishable in nature. An attempt has been made to preserve chunks of mutton in the convenience curry form using various hurdles.

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Fresh goat meat collected within 3-4 h after slaughter of the animal from a local shop of Mysore was washed with clean water, dipped in 2% acetic acid solution for 3 min. to reduce surface contamination of microorganisms and finally washed with clean water. Boned lean meat was cut into a small chunk of size of 2 cm × 1 cm × 1 cm approximately. The pieces were kept for equilibration at 5°C for 20 h in a marinating mixture containing common salt, citric acid, potassium sorbate, BHA and water at levels of 2%, 0.2%, 0.05%, 0.002% and 2.5%, respectively on fresh meat weight basis. Common salt was added as a preservative as well as a humectant. Citric acid and BHA were added as an acidulant and an antioxidant, respectively. Potassium sorbate used as an antimycotic agent is effective in a slightly reduced pH and at the desired moisture level of 35-40% of the product. It was steam-cooked for 12 min. under pressure in a kitchen pressure cooker of 12 L capacity. The chunks were dehydrated for 2 h in a cross-flow cabinet drier maintained at 45°C. The dried

TABLE 1. RECIPE FOR SHELF-STABLE SPICE PREPARATION

Ingredients	Quantity in gram (for 10 kg boned meat)
Onion	2800
Ginger	390
Garlic	360
Dalda	560
Groundnut oil	1000
Chilli powder	110
Turmeric powder	45
Coriander powder	55
Cumin powder	55
Pepper powder	17
Cinnamon powder	17
Clove powder	17
Large cardamom powder	17
Small cardamom powder	17
Common salt	220
BHA	1.12

TABLE 2. EFFECT OF TYPE OF PACKAGING AND STORAGE TEMPERATURE ON % MOISTURE, % FAT AND pH OF THE STABILIZED MUTTON CURRY [n = 6]

		Initial			After 4 months		
Packaging	Temperature	Moisture, %	pH	Fat, %	Moisture, %	pH	Fat, %
PP	RT	37.68 ± 0.24	5.58 ± 0.04	33.15 ± 0.78	35.83 ± 0.38	5.69 ± 0.05	32.31 ± 0.86
	CT	37.68 ± 0.24	5.58 ± 0.04	33.15 ± 0.78	36.61 ± 0.29	5.65 ± 0.03	32.88 ± 0.60
PFP	RT	37.68 ± 0.24	5.58 ± 0.04	33.15 ± 0.78	36.42 ± 0.33	5.67 ± 0.05	32.65 ± 0.62
	CT	37.68 ± 0.24	5.58 ± 0.04	33.15 ± 0.78	37.37 ± 0.29	5.61 ± 0.04	32.92 ± 0.52

Each value corresponded to Mean ± Standard deviation; RT= Room Temperature; CT = Chill Temperature

TABLE 3. EFFECT OF TYPE OF PACKAGING AND STORAGE TEMPERATURE ON % FFA, PV AND TBA OF THE STABILIZED MUTTON CURRY [n = 6]

		Initial			After 4 months		
Packaging	Temperature	FFA	PV	TBA	FFA	PV	TBA
PP	RT	1.53 ± 0.11	14.59 ± 3.71	0.30 ± 0.16	5.44 ± 0.24	56.05 ± 4.70	0.55 ± 0.17
	CT	1.53 ± 0.11	14.59 ± 3.71	0.30 ± 0.16	4.88 ± 0.10	22.01 ± 3.10	0.35 ± 0.16
PFP	RT	1.53 ± 0.11	14.59 ± 3.71	0.30 ± 0.16	5.09 ± 0.18	33.15 ± 3.22	0.43 ± 0.19
	CT	1.53 ± 0.11	14.59 ± 3.71	0.30 ± 0.16	3.16 ± 0.18	17.72 ± 2.54	0.34 ± 0.14

Each value corresponded to Mean ± Standard deviation; RT= Room Temperature; CT = Chill Temperature
FFA = % as oleic acid; PV = milliequivalent peroxide/kg); TBA = mg malonaldehyde

TABLE 4. EFFECT OF TYPE OF PACKAGING AND STORAGE TEMPERATURE ON SENSORY QUALITY PARAMETERS OF THE STABILIZED MUTTON CURRY [n = 15]

Storage period, months	Packaging	Temperature	Colour and appearance	Texture	Flavour	After-taste	Rancidity	Overall acceptability
0	PP	RT	7.56 ± 0.66	7.50 ± 0.71	7.27 ± 0.75	6.97 ± 1.01	7.40 ± 0.92	7.43 ± 0.50
		CT	7.56 ± 0.66	7.50 ± 0.71	7.27 ± 0.75	6.97 ± 1.01	7.40 ± 0.92	7.43 ± 0.50
	PFP	RT	7.56 ± 0.66	7.50 ± 0.71	7.27 ± 0.75	6.97 ± 1.01	7.40 ± 0.92	7.43 ± 0.50
		CT	7.56 ± 0.66	7.50 ± 0.71	7.27 ± 0.75	6.97 ± 1.01	7.40 ± 0.92	7.43 ± 0.50
4	PP	RT	7.27 ± 0.73	7.53 ± 0.47	6.33 ± 0.87	6.80 ± 0.93	5.97 ± 0.77	6.60 ± 0.63
		CT	7.33 ± 0.60	7.40 ± 0.60	7.20 ± 0.63	6.93 ± 0.50	6.60 ± 0.73	7.00 ± 0.40
	PFP	RT	7.47 ± 0.66	7.53 ± 0.63	6.60 ± 0.47	6.33 ± 0.67	6.06 ± 0.57	6.96 ± 0.33
		CT	7.53 ± 0.80	7.40 ± 0.73	7.20 ± 0.60	6.73 ± 0.67	7.03 ± 0.37	7.20 ± 0.47

Each value corresponded to Mean ± Standard deviation; RT= Room Temperature; CT = Chill Temperature

chunks were then mixed with a shelf stable spice preparation (Table 1) in hot condition, cooled and packaged in 45 GSM paper - 20 μ aluminium foil - 37.5 μ polyethylene laminate (PFP) and 50 μ polypropylene film (PP) pouches.

The packaged samples were stored at room temperature of 27 ± 2°C (RT) as well as at chill temperature of 3 ± 2°C (CT) for storage stability studies, which were carried out by analysing various quality parameters such as moisture content, fat content, FFA and PV by AOAC (1990); TBA by distillation method; pH by pH meter WTW pH 330, W-B shear value by Warner-Bratzler Shear Apparatus (John Chatillon and Sons); sensory evaluation by semi-trained panelists of the laboratory using a 9-point Hedonic scale (Amerine et al. 1965) and microbiological quality by total plate counts (TPC) and yeast and mold counts (Y & M) (APHA 1992). The tabulated values were mean ± standard deviation of six observations in case of moisture content, pH, fat content, FFA, PV, TBA, TPC and Y & M; 10 observations in case of W-B shear and 15 observations in case of sensory scores.

The product under study having 37.68% moisture and 5.58 pH was stable for four months at ambient temperature and more than six months at refrigeration temperature. There was no marked change in moisture content of the product during storage. There was an increase in pH of the product under all storage conditions, which is supported by the findings of Yadav and Sanyal (1999). The increase in pH of the products might be due to the production of amines from meat proteins during the course of storage. A decrease in fat content of the product on storage was observed under both the packagings and at both the temperatures (Table 2). Similar observations have been made by Kesava Rao and Kowale (1991) as well as Mark and Ubu (1991). It was further noticed that the increase in pH value and the decrease in fat content were more in the case of PP packaging and room temperature storage than for PFP packaging and refrigerated storage. Rancidity as evidenced from FFA, PV and TBA values increased in both the packaging and storage conditions. The products under both the packaging and storage conditions

showed greater increases in PVs than in FFA and TBA values. Oxidation of fat as indicated by PVs and TBA values was more in case of product packaged in PP than those in PFP pouches and in case of ambient temperature storage than refrigeration storage. Change in % FFA was not distinctive with respect to packaging materials and storage temperature (Table 3). The microbiological quality of freshly processed curry (TPC-3.33 log cfu/g, Y & M-3.10 log cfu/g) was satisfactory and it improved further on storage. This is called auto-sterilization that takes place in hurdle-preserved foods during storage, especially at ambient temperature (Leistner 1995). It was observed that the product in PP pouches after 4 months of storage at both the temperatures has got more Y & M counts (RT - 2.24 log cfu/g, CT-1.86 log cfu/g) and less TPC (RT - 3.02 log cfu/g, CT-3.02 log cfu/g) than those in PFP pouches (Y & M at RT-2.04 log cfu/g, Y & M at CT-1.81 log cfu/g, TPC at RT-3.30 log cfu/g, TPC at CT-3.17 log cfu/g). Mean values of W-B shear both along and across the length of muscle fibre in chunks of the freshly processed curry were 0.0634 and 0.1387 kg/cm, respectively, which after 4 months of storage at RT, decreased to 0.0515 and 0.1129 kg/cm in the case of product in PP and 0.0581 and 0.1203 kg/cm in the case of product in PFP packaging. On the other hand, these values were 0.0600 and 0.1274 kg/cm in the case of PP and 0.0612 and 0.1326 kg/cm in the case of PFP, respectively at the end of 4 months storage at CT. The recorded data implied that storage at ambient temperature and packaging in PP had more softening effect on muscle than storage at CT and packaging in PFP respectively. The decreases in shear values during storage might be attributed to the softening of muscle fibres and connective tissues. Himanish (1999) observed reduction in shear value of muscle on storage. The freshly prepared product recorded a very good organoleptical score on a 9-point Hedonic scale. There was almost no observable change in mean score values for texture. Mean scores for colour and appearance and after-taste declined marginally. The samples demonstrated lower average scores for both flavour and rancidity after 4 months of storage. Flavour changes were more in case of room temperature storage for either of the packaging materials but no trend was established for decrease in after-taste score during storage with respect to temperature of storage and type of packaging materials (Table 4).

Instant mutton curry developed by hurdle technology was microbiologically safe and organoleptically acceptable. It had a very good shelf life of four months at ambient temperature and a brief cooking time of 14 min. in boiling water. For the product, the PFP pouch was the most suitable packaging, which was easy-to-handle and transport too. During storage, texture and microbial quality of the product had improved over the course of time. This convenient traditional meat product may find a place in the shelves of grocers, shops, hotels and restaurants as well as in military kitchens.

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RESEARCH NOTE

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Preparation and Organoleptic Evaluation of Soy-blended Food Products

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Full fat soyflour was blended with cereal or pulse flours to make a variety of common Indian traditional products, namely *chapati, pakoda, burfi* and extruded snacks. These recipes were evaluated by a trained panel of judges for their acceptability and other quality parameters. From the mean score values, it was observed that all the products were liked and were acceptable by the judges. These products were devoid of off flavour and had acceptable characteristics. Analysis of variance (ANOVA) indicated siginificant differences among all the products at 5 and 1% levels of significance, while the disagreement among the judges for all the characteristics was not significant, while the disagreement among the judges for all the characteristics was not significant at 5 and 1% levels, thereby confirming the homogeneity of panelists.

Keywords : Soybean, Sodium bicarbonate, Full-fat soyflour, Soy-blended products, Organoleptic evaluation.

The search for a new unconventional source of proteins to meet the requirement of ever expanding population of India is the dire need of the present day. Soybeans with 40% proteins and 20% oil have a great potential in solving the problems of protein and calorie malnutrition. Starting from 1965, efforts were being made to produce soybeans in dry farming areas. Madhya Pradesh is the leading State in India with 3.95 million hectares under soybean cultivation. However, soybeans have not gained a place in the diets of rural people, though there is an ever growing demand for soy products in the market (Gandhi 1988).

The idea of substituting soybeans for common beans/ pulses appears sound, especially in the countries in which the consumption of the latter is considerable. This has wide application in India, where common beans together with wheat and rice form the staple diet of the majority of the population (Deshpande 1999).

Full fat soyflours were blended at different proportions with cereal, millet or pulse flours to make a wide range of traditional dishes. The present investigation was carried out to study the organolentic qualities of various indigenous products prepared from soy blends.

The soybean variety 'JS-335' grown in the experimental farm of the Central Institute of Agricultural Engineering, Bhopal, was used for the present study.

The whole sound soybeans were cleaned of dirt, foreign matter and brokens. The beans (8% moisture on wet basis) were cracked with a mini grain mill and the hulls were removed with a hand winnower. The split pulses were filled in cloth bags to one third of their fullest capacity and soaked in water, containing 1% (w/v) sodium bicarbonate at ambient temperature for 4 h. The beans were conditioned to 52% moisture on wet basis. The beans to water ratio was 1:3. The addition of sodium bicarbonate aided the improvement of beany or greasy flavour. The soaked beans were cooked in boiling water (100°C) for 15-20 min to detoxify the beans. Later, the bags were lifted up and allowed to drain for a few minutes. The cooked beans were spread in single layers on black polyethylene surfaces and dried for 32-36 h to bring the moisture level again to 8%. The size reduction was undertaken in a mini grain mill. Thus, the product was made ready for use (Gandhi et al. 1983).

Products prepared from different soy blends : Full fat soyflour was blended with cereal or pulse flours to make a variety of common Indian traditional products namely, *chapati, pakoda, burfi* and soy blend extruded snacks. Organoleptic evaluation was conducted on these products.

Soy-blended chapati (Roti) : Wheat is a staple food of villagers and consumed during meals in the form of *chapati*. Hence, wheat flour was blended with full-fat-soyflour by dry mixing in the proportion of 90:10. These flours were sifted and mixed thoroughly. Salt was added and kneaded into a dough with required quantity of water. It was divided into three equal proportions and rolled out into *chapatis.* They were baked on a hot pan for about 2 min and spread with ghee. A sample of *chapati* prepared from only wheat flour was used as reference for comparison.

*Bengal*gram (chickpea) is a widely grown pulse crop of the study area with a high protein content of about 22%. This is most commonly consumed by the villagers in different forms such as *pakoda*, and cooked dehusked split *dhal* (broth).

Pakoda : It was prepared using the following ingredients: Soyflour 20 g ; chickpea flour 80 g ; chopped onions 20 g ; chopped green chillies 10 g ; cumin seeds 1 teaspoon ; paprika powder 1 teaspoon ; salt to taste ; and fat for frying. The product contained energy 326 kcal, proteins 17.2 g, fat 18.08 g, carbohydrates 36.14 g , ash 3.28 g and moisture 25.3 g per 100 g of products.

Method of preparation : Soy flour and chickpea flour were sieved. Chopped onions, green chillis, cumin seeds, paprika powder and salt to the flour were added along with water in required quantity to bring the batter to the consistency required for *pakoda* making. Oil was then heated and batter

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in the form of *pakoda* was fried till deep brown. *Pakoda* prepared using the same procedure from only chickpea flour was used for comparison.

Burfi : This was prepared using the following ingredients: Soy flour 20 g; Chickpea flour 80 g ; canesugar 50 g ; hydrogenated fat 50 g. The product thus prepared contained energy 511 kcl, proteins 12.1 g , fat 28 g , carbohydrates 46.3 g , ash 4.12 g and moisture 9.48 g per 100 g of products.

Method of preparation : The sieved soyflour and chickpea flour were mixed well and fried in hydrogenated fat till golden yellow. Water was added to cane sugar and two thread consistency syrup was prepared. Syrup was added to the fried flour and cooked for 2 min. Mixture was then poured on a greased tray with ghee and allowed to settle for 2 h. and cut into the desired shape. The *burfi* prepared employing the same procedure but using only chickpea flour was used as a reference for comparison.

Soy blend extruded snacks : Extruded snacks were prepared using different ingredients as follows : Soy *dhal* 10 g; rice 200 g ; sago 200 g ; cumin seeds 3 teaspoons, salt to taste. The product contained energy 365 kcal, proteins 11.8 g, fat 4.5 g, carbohydrates 70.7 g, ash 4.3 g and moisture 9.7 g per 100 g of products.

Method of preparation : Soy *dhal*, rice and sago were milled in a hammer mill and sieved. Salt and cumin seeds were added to the flours. Water at the rate of 400 ml/100 g of blend flour was added and thoroughly mixed to form a slurry. The slurry was cooked in a container for 12-15 min to obtain a thick paste. The cooked material was then pressed in a household *Sev* machine (extruder) to get the desired shape of snacks. The snacks, thus, produced were sun-dried and could be used as and when needed after deep-frying in vegetable oil. Extruded snacks prepared using the same technique using only sago and rice flour was used for comparison.

Sensory evaluation of soy blends : Sensory evaluation was conducted on different recipes prepared from soy blends by a panel of 9 trained judges (ISI 1971). The various characteristics like taste or flavour, feeling or texture, colour or appearance and general acceptability for each of the products were assessed using a 9-point Hedonic scale, indicating 9 = excellent, 8 = very good, 7 = good, fair=6 below good and above 5 = fair below fair and above 4 = poor, 3 = poor, 2 = very poor, and 1 = extremely poor. Analysis of variance was used to test the difference between the treatments (ISI 1975).

Organoleptic evaluation of soy-blended food products : The products viz, *chapati, pakoda, burfi* and extruded snacks prepared were organoleptically evaluated by a panel of trained judges for acceptability and other quality parameters. The scores were allotted to different quality characters as compared to the original recipe without soyflour, assuming the highest acceptability for them as per the standard procedure (IS:6273, 1971) on a 9 - point Hedonic scale. The score of 5 was considered as acceptable. The mean scores awarded were computed. The mean score values of the different soy-blended food products for different characters such as taste, flavour are given in Table 1.

The mean scores for all the quality characters and general acceptability were more than the minimum acceptable score of 5. The results, thus, indicated that all the products

TABLE 1. MEAN SCORES OF SENSORY PANEL JUDGES FOR THE CHARACTERISTICS OF DIFFERENT SOYBEAN PRODUCTS

Character	Products			
	Chapati	*Pakoda*	*Burfi*	Extruded snack
Taste	7.27	7.38	8.50	7.16
Flavour	7.00	7.05	8.16	7.27
Colour	7.50	7.88	8.00	7.55
Texture	6.77	7.55	8.11	7.11
Appearance	7.16	7.88	7.94	7.77
Overall acceptability	7.05	7.38	8.16	7.11

TABLE 2. ANALYSIS OF VARIANCE DATA FOR OVERALL ACCEPTABILITY AND OTHER QUALITY ATTRIBUTES OF DIFFERENT SOY-BLENDED PRODUCTS

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-ratio
Taste				
Judges	8	4.76	0.59	0.786^{ns}
Products	3	10.94	3.64	4.815*
Error	24	18.18	0.75	-
Total	35	33.88	-	-
Flavour				
Judges	8	6.18	0.77	1.546^{ns}
Products	3	7.13	2.37	4.764*
Error	24	11.98	0.49	-
Total	35	24.29	-	-
Colour				
Judges	8	1.38	0.17	0.336^{ns}
Products	3	1.79	0.59	1.161^{ns}
Error	24	12.38	0.51	-
Total	35	15.55	-	-
Texture				
Judges	8	2.30	0.28	0.827^{ns}
Products	3	385.89	128.63	369.22*
Error	24	8.36	0.34	-
Total	35	397.15	-	-
Appearance				
Judges	8	1.13	0.14	0.23^{ns}
Products	3	3.47	1.15	1.91^{ns}
Error	24	14.52	0.60	-
Total	35	19.12	-	-
Overall acceptability				
Judges	8	6.13	0.76	1.09^{ns}
Products	3	7.07	0.76	3.35**
Error	24	16.86	0.70	
Total	35	30.06	-	

*Significant at 1 % level, ** Significant at 5 % level, ^{ns} non significant

prepared from soyflour, when blended with wheat flour, chickpea flour, rice and sago were well liked. These products were devoid of off flavour and possessed acceptable characteristics. The results are in accordance with Deshpande (1990), who obtained similar results, while evaluating different products (*dhal* and ready-to-eat crispies), prepared from full fat soyflakes in combination with pigeonpea *dhal* and rice flakes. Further, Vijayalakshmi and Vaidehi (1982) also obtained similar results, while evaluating *Tofu* blended with chickpea flour. The results are also in agreement with the earlier studies carried out by Patil et al (1989) regarding organoleptic evaluation of various snacks prepared from combinations of soyflakes and rice flakes.

A separate analysis of variance (ANOVA) was done for each character, taste, flavour, colour, texture, appearance and overall acceptability from every individual score of taste panel for different soy blend products (ISI 1975), using a computer programme specially developed for this purpose. This analysis was carried out to find out the differences among the charactertistics and panelists for the various products and the results are presented in Table 2.

The results revealed that the differences among the various products were significant for taste, texture at 1 % level of significance, whereas for overall acceptability, the difference was observed to be significant at 5 % level. For some of the quality characters such as, colour and appearance, the differences were found to be non-significant (Table 2). This may be due to the use of similar cooking methods, which are used in traditional preparations (standardized recipe). The disagreements among the judges for all the characteristics were not significant at 5 % and 1% levels of significance.

Thus, the data indicated that the source of variance among the products was due to the quality characteristics like taste, flavour, texture and overall acceptability. This was obvious due to the inhibitory attitude of people towards soybean due to its inherant characteristic qualities. It was also expected to be a new food product that deviated from the conventional foods. Further, the variances in scores by judges did not exist, indicating that the group of panelists was homogeneous and all the soy blend preparations were acceptable. The results were also in complete confirmity with the earlier work carried out by Gandhi et al (1983), Shukla and Saxena (1988) and Sahay and Kachru (1988), who obtained similar results, while evaluating a variety of Indian traditional products blended with full fat soyflour.

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Studies of Fortification of Sour Whey Concentrate in *Chakka* for Preparation of *Shrikhand*

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Addition of 5 % sour whey concentrate in *chakka* (both from cow's milk) increased the yield of *shrikhand* by 5 % over that by traditional method. This addition, however, did not bring about any change in the physical attributes of *shrikhand*. Addition of whey concentrate to more than 5 % level brought about deterioration in the quality of *shrikhand* in terms of flavour, consistency, body and taste.

Keywords : Whey concentrate, Fortification, *Chakka, Shrikhand,* Sensory qualities.

Shrikhand manufacturing practices involve partial loss of whey solids. In order to balance the nutritional loss of whey solids and to use whey solids economically, the use of whey, whey concentrate and whey protein concentrate is becoming more common.

The food manufacturing industries have come to realize that milk proteins in general and whey proteins, in particular, have a great potential to improve the quality of food products. Kester and Richardson (1984), Kim et al (1989), Morr and Ha (1993) and Jayaprakash et al (1997) studied the potential of these proteins. Jayaprakash and Brueckner (1999) reported whey, whey concentrate and whey protein concentrate as the potential functional ingredients for the food industry.

In the present study, an attempt was made to explore the use of whey concentrate in *shrikhand* preparation and its effect on yield as well as sensory and chemical characteristics.

Cow's milk (5 litre) after boiling for 10 min and then cooling was used to obtain *chakka* for the usual method of *shrikhand* preparation. The yields of *chakka* and whey obtained were 350 g and 650 ml, respectively. The whey was transferred into a stainless steel pan (5.8 ml/cm^2) and concentrated to about 1/5th proportion by raising the temperature to 70°C. The 350 g *chakka* thus obtained was mixed with 40% sugar and blended with nutmeg powder (0.5 g) and well kneaded to prepare *shrikhand*. This was treated as control (T_0). The *chakka* (350 g) obtained in other treatments was mixed with whey concentrate along with sugar (40%) and nutmeg powder (0.5 g) in the following proportions in the other treatments : T_1 = 5 % whey concentrate ; T_2 = 10 % whey concentrate; T_3 = 15 % whey concentrate and T_4 = 20 % whey concentrate

The *shrikhand* so prepared was subjected to sensory evaluation by a panel of 10 semi-trained judges on a 9-point Hedonic scale (Gupta 1976). The data were analyzed statistically, following Randomized Block Design as per the method of Panse and Sukhatme (1985) and the trial was repeated four times. The *shrikhand* prepared in each treatment was subjected to chemical analysis for various characteristics as per the method of Bureau of Indian Standards (BIS 1981).

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Mixing of whey concentrate at 5 % level in the *chakka* to prepare *shrikhand* showed an increase in the yield by about 5 % as compared to control (Table 1) and brought about negligible changes in the physical attributes of *shrikhand*. The increase in yield in proportion to increase in whey concentrate from 10 to 20 % fortification, was, however, negligible. Addition of whey concentrate beyond 5 % level was not found much useful, as it caused deterioration in the quality of *shrikhand* with respect to flavour, consistency and taste (Table 2).

The sensory evaluation data (Table 2) showed that *shrikhand* fortified with whey concentrate was more acidic as compared to the control. There were not much differences in the flavour scores of all the blends. The consistency and body characteristics of whey concentrate-fortified *shrikhand* got reduced and became more loose as compared to control. The

TABLE 1. EFFECT OF MIXING WHEY CONCENTRATE IN THE *CHAKKA* ON YIELD OF *SHRIKHAND*

Addition of whey concentrate, %	Concentrated whey added, g	Yield of *shrikhand*, g
0 (Control)	-	490.0
5	17.5	514.5
10	35.0	539.0
15	52.5	563.5
20	70.0	580.0

Each value is the average of three replications

TABLE 2. SENSORY SCORES OF *SHRIKHAND* AS INFLUENCED BY FORTIFICATION OF WHEY CONCENTRATE IN *CHAKKA*

Addition of whey concentrate, %	Flavour	Consistency	Body	Taste
0 (Control)	6.9	7.1	7.4	7.6
5	6.5	6.6	6.9	7.2
10	6.5	6.4	6.8	6.3
15	6.2	6.2	6.7	6.0
20	6.1	6.1	6.3	5.3
SE ±	0.35	0.2	0.2	0.25
CD at 5 %	1.00	0.6	0.6	0.70

Each value is the average of four replications

TABLE 3. EFFECT OF FORTIFICATION OF WHEY CONCENTRATE ON COMPOSITION OF *SHRIKHAND*

Fortification levels, %	Constituents, %						
	Mois-ture	Fat	Protein	Lactose	Total solids	Ash	Acidity
0 (Control)	49.86	13.5	7.38	5.3	50.14	0.73	1.0
5	49.30	12.8	7.45	5.6	50.70	0.87	1 1
10	49.12	12.1	7.49	5.9	50.86	1.21	1.1
15	48.88	10.7	7.58	6.2	51.12	1.48	1.3
20	48.46	9.7	7.59	6.3	51.54	1.63	1.4

Each value is the average of four replications

taste of *shrikhand* prepared from 5% whey concentrate-fortified *chakka* and control was very much alike, but taste deteriorated and rated unsatisfactory with higher levels of fortification of whey concentrate.

The chemical composition of *shrikhand* prepared from the conventional method without whey concentrate and *shrikhand* fortified with whey concentrate at varying levels (Table 3) showed that addition of whey concentrate in the *chakka* increased the proteins, lactose and ash contents to some extent in the final product. Jayaprakash and Brueckner (1999) reported that the whey protein concentrate containing 35% proteins could be used commonly as replacement for milk solids-not-fat because of the cost advantage. The actual legal requirements currently allow 25% substitution of whey solids for milk solids-not-fat for frozen dairy products (Kelly 1986). A frozen yoghurt has been successfully prepared by replacing 50 % of skim milk solids with whey protein concentrate by Opdahl and Baer (1991) and Jayaprakash (1998). They further reported that the resultant product had better body and texture and had a consistency of ice cream.

It may be concluded that whey concentrate can be added to *chakka* at 5 % level to prepare *shrikhand* without any adverse effects on sensory and chemical qualities of *shrikhand.*

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RESEARCH NOTE

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Comparative Performance Evaluation of Different Drying Methods for Chillis

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A study was conducted to evaluate the performance of different drying methods, namely open sun-drying on cemented floor, green house-type solar drying (with natural ventilation system), solar cabinet drying (natural convection type) and mechanical drying for both unpunched and punched fresh red chillis of 'Jwala' variety. The moisture content versus time and drying rate versus moisture content relationships were determined. The quality parameters like pungency and colour were estimated and the economics of drying were calculated both for unpunched and punched chillis dried by the above methods. It was observed that the time taken for drying was minimum for mechanical drying, followed by solar cabinet drying, green house-type solar drying and opens sun-drying. The overall quality was found to be better in mechanical drying, followed by green house-type solar drying, solar cabinet drying and open sun-drying. The green house solar drying was most economical, followed by solar cabinet drying, mechanical drying and open sun-drying.

Keywords : Drying of chillis, Sun-drying, Green house-type drying, Mechanical drying, Solar cabinet dryer, Overall quality, Economics.

India is known for the production and export of spices since time immemorial. Among the spices, chilli being one of the major seasonal spices is consumed in excess of all other spices. The initial moisture contents of fresh red chillis range from 300 to 400 % (db), which are too high for their processing and storage. Therefore, reduction of moisture contents of chillis to a safe level of 8-9% (db) becomes necessary before their processing and storage.

In the major chilli producing States of India, drying is carried out during the rainy months of November-March, resulting in large quantitative and qualitative losses. Traditionally, chillis are sun-dried on mud/cement floors, roadside, roof tops etc. An improved method is, therefore, essential to reduce these losses.

The four different methods were used namely, open sun-drying on cemented floor, green house-type solar drying (with natural ventilation system), solar cabinet drying (natural convection type) and mechanical drying (tray type). Both, unpunched and punched chillis of 'Jwala' variety was used in the above methods.

Illyas et al (1987) developed a chilli machine capable of punching of chillis at the rate of 10 kg/h. It was found that the punched chillis dried faster than unpunched ones. Chandy et al (1992) conducted an experiment to evaluate the effect of pricking and destalking and chopping on the drying characteristics of fresh red chillis. The pre-treated chillis were dried in the sun and in mechanical dryer. They concluded that chopping treatment excelled overall in terms of time of drying and energy required. Central Institute of Agricultural Engineering, Bhopal developed a solar cabinet dryer (30-50 kg) for dying of perishable, semi-perishable and wet processed food materials. It was reported that it took 4-7 days/ batch to dry chillis from 70-80% (wb) to a equilibrium moisture content. Juliet (1992) used the concept of green house-type solar dryer for drying pigeonpea. A simulation model to predict the drying behaviour of the product in the forced ventilation solar system was developed. The model suggested that even under unfavourable conditions of low temperature, high relative humidity and low radiation, the drying took place in the solar dryer, at a slower pace. It was further observed that a green house-type solar dryer could provide a viable alternative to traditional open sun-drying. Gangopadhyay and Chanderi (1979) dried pricked and unpricked peas in a through- flow drier and fluidized bed dryer and reported that unpricked peas took twice the time that was needed for the pricked ones.

For conducting the experiments, fresh red chillis of 'Jwala' variety were obtained from a farmer and were graded for shape and colour and unripe, spoiled chillis were removed. Half the chillis were punched in chilli punching machine, which was developed in the Division of Agricultural Engineering, Indian Agricultural Research Institute, New Delhi. For punching operation, the fresh red chillis were placed in the tray of the chilli punching machine and the needle assembly was brought down with the help of hand lever. About 20 holes per chilli pod were obtained in four punching operations.

Drying : Both unpunched and punched chillis of 2 kg each were taken for all the drying methods. The ripened red chillis with an initial moisture content of 300% (db) were dried to the final moisture content of 8-9% (db).

Sun-drying : The chillis were allowed to dry in a single layer by spreading on cemented floor. The time of drying was from 9 a.m. to 3 p.m. After a day, the dried chillis were packed in polythene bags (guage 200), which were moisture proof. The temperature and relative humidity of the ambient were noted after every 2 h with the help of a thermometer and sling psychrometer, respectively. The moisture contents of the chillis were determined after every 6 h, till an optimum moisture content of 8-9%(db) was obtained. All these experiments were replicated three times.

Solar cabinet drying : Both punched and unpunched chillis were spread on two different trays separately in a single

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layer in the solar cabinet dryer (natural convection type) and allowed to dry. The essential environmental parameters like temperature, relative humidity, solar radiation, air flow rate inside and outside the solar cabinet dryer were measured after every 2 h along with total sunshine hours in a day. The moisture contents of chillis were determined after every 6 h, till an optimum moisture content of 8-9% (db) was obtained. All these experiments were replicated three times.

Greenhouse-type solar dryer : The green house-type, which was used for the experiment, was developed in the Division of Agricultural Engineering, Indian Agricultural Research Institute, New Delhi. A continuous air inlet of 1 ft width was provided on one side of the green house along the bottom of the vertical wall for the entry of natural air. A chimney of 6.06 m height was installed with the green house at the other end of natural ventilation. Excepting this, all other sides of the green house were fairly airtight. Hence, natural air entered at one end of the green house and the moisture laden air escaped through chimney, which provided suitable condition for drying. Drying racks and trays were provided in the greenhouse for drying of chillis.

For conducting the experiment, both punched and unpunched chillis were spread on trays in a single layer and placed over the racks in the greenhouse for drying. The essential study parameters were measured after the equal time intervals. The moisture contents of the chillis were determined after every 6 h, till the optimum moisture content of 8-9 (db) was obtained. All these experiments were replicated three times.

Mechanical drying : The mechanical dryer (tray type), which was used for the experimental study, was designed and developed in the Division of Agricultural Engineering, Indian Agricultural Research Institute, New Delhi. Unpunched chillis of 2 kg at the initial moisture content of 30% (db) were spread uniformly in two trays and were dried with heated air at 45°C with the airflow rate of 0.019 m^3/min. The moisture contents of the chillis were determined after every 2 h, till it attained an optimum moisture content of 8-9% (db). Similar trials were also undertaken with unpunched chillis (2 kg) and all the experiments were replicated three times. Energy consumed in drying the chillis in the dryer was determined with the help of an energy meter connected across the tray dryer.

Quality test procedure

The chilli specimens so dried were tested for pungency and colour as described below.

Pungency test : Four grams of chilli powder were extracted with acetone, till a colourless acetone solution was obtained. The volume was made up to 100 ml with acetone. The extract was kept for 3 h at room temperature. After 3 h, 5 ml of acetone extract was taken in a beaker and was heated on water bath a till fully dry. To this solution, 5 ml of 0.1 N NaOH solution was added, followed by 3% phosphomolybdic acid solution and was kept at room temperature for 1 h. Finally, optical density values were measured at 650 mμ with the help of a spectrophotometer. The sample with the highest optical density was considered to be containing more capsaicin and hence, more pungent.

Colour test : The colour of chilli dried by the different methods was determined by matching with the colour of munsel colour chart.

Specimen evaluation chart was used for numerical scoring for colour quality (Hedonic scale): A set of panelists was asked to evaluate the colour parameters of the dried chillis on a 9-point scale and scores obtained by different samples were analysed statistically.

Comparison of drying characteristics : The reduction in moisture content with reference to the time spent in sun-drying, green house-type solar drying, solar cabinet drying and mechanical drying is shown in Figs. 1-4 and in Table 1 for unpunched and punched chillis. Open sun-drying took the maximum time of 150 h for unpunched and 102 h for punched chillis to dry to the moisture content of 8-9% (db). This might be because the experiment was conducted in the months of December to March during which the prevalent ambient temperature varied from 9-20°C with a high R.H. This did not provide favourable conditions for rapid removal of moisture. Green house-type solar dryer took 90 h for the unpunched and 66 h for the punched chillis, showing a saving of around 40% time in comparison to sun-drying. This was due to the fact that the temperature inside the greenhouse was 6-12°C higher and R.H. 5-6 % less than the ambient. Solar cabinet took 54 h for the unpunched and 36 h for the punched chillis, which were 60% and 40% less than the sun-drying and green house-type solar drying, respectively. This was due to the reason that the

TABLE 1. AVERAGE DRYING TIME, MEAN OPTICAL DENSITY, MEAN COLOUR VALUES, MEAN SENSORY SCORES AND AVERAGE COST OF DRYING OF CHILLIS BY DIFFERENT METHODS

Methods	Average drying time, h		Mean optical density		Mean colour value		Mean sensory score		Average cost of drying (Rs./kg)	
	Unpunched	Punched	Unpunched	Punched	Unpunched	Punched	Unpunched	Punched	Unpunched	Punched
Sun-drying	150	102	0.4903	0.4250	10R, 4/6	10R, 5/6	4.0	3	20.87	14.19
Greenhouse-type solar drying	90	66	0.5953	0.5647	7.5R, 3/8	7.5R, 4/8	8.5	8	2.90	2.13
Solar cabinet drying	54	36	0.5760	0.5310	7.5R, 4/6	7.5R, 5/6	8.0	7	3.10	2.17
Mechanical drying	26	16	0.6213	0.5780	5R, 4/8	5R, 4/6	9.5	9	4.98	3.06

temperature in the solar cabinet dryer was 19-20°C higher and the relative humidity 10-30% less than the ambient, which provided favourable conditions for drying.

Mechanical drying took 26 h for the unpunched and 16 h for the punched chillis, which was substantially lesser than other methods. This was because the temperature was maintained at 45°C in the dryer.

Quality evaluation test

Pungency test : The capsaicin content of the chillis dried

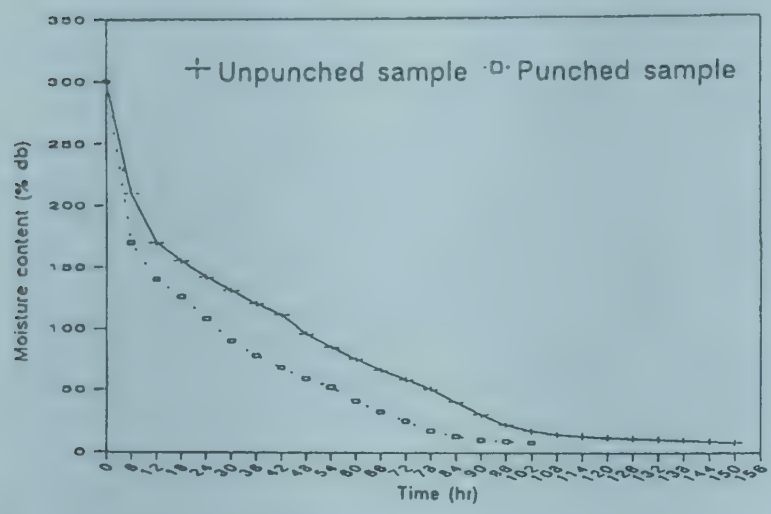

Fig. 1. Moisture content vs. time for both unpunched and punched chillis in sun-drying

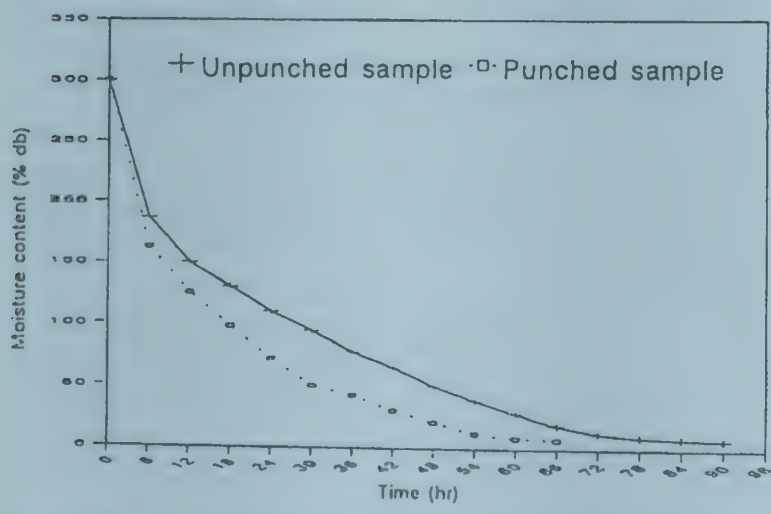

Fig. 2. Moisture content vs. time for both unpunched and punched chillis in greenhouse-type solar drying

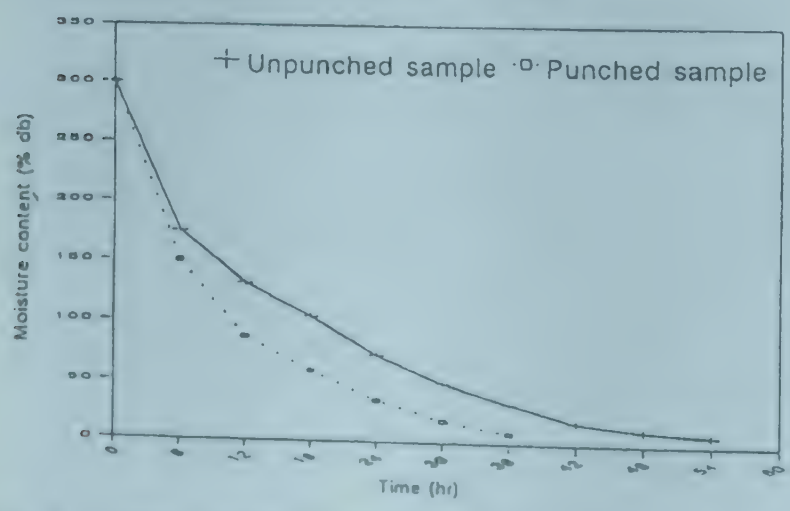

Fig. 3. Moisture content vs. time for both unpunched and punched chillis in solar cabinet drying

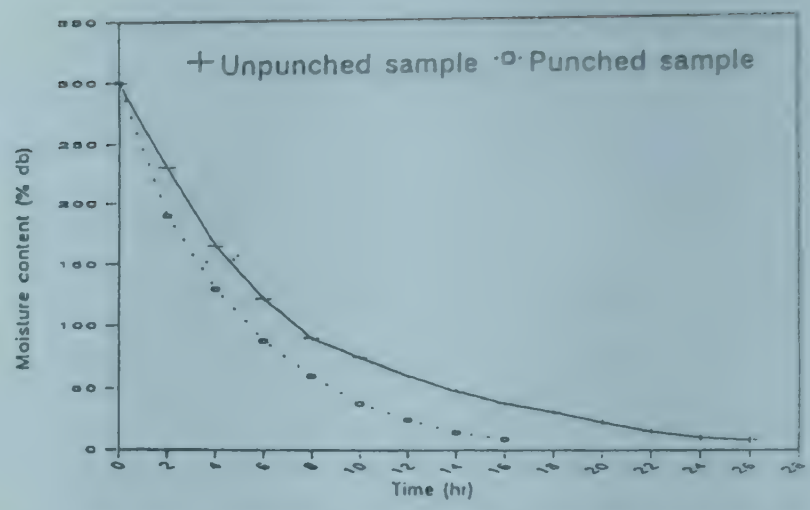

Fig. 4. Moisture content vs. time for both unpunched and punched chillis in mechanical drying

by different methods with punching and without punching as estimated in terms of optical density and the results are presented in Table 1. The results indicate that the capsaicin content of mechanically dried samples was the highest, followed by green house-type solar drying, solar cabinet drying and sun drying. Thus, pungency in mechanically dried samples was best, followed by green house-type solar drying, solar cabinet drying and sun-drying.

Colour test : The values obtained by matching chilli samples dried by different methods are presented in Table 1. The redness of the sample was expressed as 5R>7.5>10R. The 5R, 7.5R, 10R are known as "hue",which is the dominant spectral colour and is related to the wave length of light. The expressions like 4/8, 4/6, 5/6 and 4/8 (Table 1) have the numerator known as "Value" and it represents the relative lightness of colours which is a function of the square root of the total amount of light reflected. The figure in the denominator is known as "Chroma" related to the purity or the strength of the spectral colour and is inversely proportional to the greyness. It was observed from the results that the colour of mechanically dried samples was the best, followed by green house-type solar drying, solar cabinet and sun-drying.

Organoleptic evaluation : The chilli samples dried by different methods were evaluated for their appearance. The results are presented in Table 1. The results indicate that mechanically dried chilli samples were the best, followed by green house-type solar drying, solar cabinet drying and was very poor in case of open sun drying.

Economics of different drying methods : The average cost of drying of different methods is presented in Table 1. It was evident from the results that the cost of drying chillis in green house-type dryer was minimum and it was maximum for open sun-drying.

It may be concluded that in all the methods of drying, the time required for punched chillis was less than the unpunched chillis. The punching operation reduced the time and cost of drying (Rs./kg) but it affected the quality (pungency and colour) of the final product. The time required for drying was the least by mechanical drying, followed by solar cabinet drying, green house-type solar drying and sun-drying. The

quality (pungency and colour) of the mechanically dried product was superior, followed by green house-type solar drying, solar cabinet drying and was poor by sun-drying. The green house-type solar drying was found to be most economical, followed by solar cabinet drying, mechanical drying and sun-drying.

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FOOD PROCESSING AND PRESERVATION : Written by G. Subbulakshmi and Shobha A Udipi. Published by New Age International (P) Ltd, New Delhi - 110 002, 2001, pp 298, Price : Rs. 165/-

Food processing transforms raw plant, animal and marine materials into intermediates or edible products through application of labour, machinery, energy and scientific knowledge. This achieves conversion of relatively bulky perishable and typically inedible food materials into more useful, concentrated, shelf stable and palatable foods or potable beverages. The food industry is challenged to produce products with longer shelf life that also possess quality attributes very near to those of native state of foods through minimising the detrimental influence of processing treatments and developing new technologies.

This book, in essence, is an attempt to consolidate in brief the knowledge gathered through ages on food processing technologies. A glance at the contents gives details of the key topics covered in 10 chapters. The book begins with introduction covering historical aspects of food production, processed foods, general principles of food preservation and speciality products for use by Defence forces. This is followed by Chapter I on Cereal Technology highlighting that half of the world's population consumes rice as staple food and cereals supply as much as 70% of the person's energy intake. Post-harvest losses, agents causing spoilage, remedial measures, milling, products / by-products, improved methods of storage are well presented.

More than 75% of legumes are consumed as *dhal. Dhal* milling is a big industry and authors have presented a good review of legume technology in Chapter 2. Emphasis is given on toxic factors in legumes, viz., β - N - oxalyl - amino - L - alanine in *kesri dhal* responsible for spastic paralysis with nervous symptoms, goitrogenic thiocyanates and isothiocyanates in rapeseeds, mustards etc., divicine and isouramil in certain variety of beans, causing haemolytic anaemia and various others. Possible methods of detoxification are discussed. Also, presented through flow diagrams are preparations of varieties of legume products, quality evaluation etc.

Groundnut, castorseed, sesame, rapeseed and linseed are the five major oilseeds produced in India, annual production amounting to 6-7% of total volume of agricultural production in the country. Chapter 3 deals with processing of oilseeds for production of oil, refining, varieties of products such as margarine, peanut butter etc. Evaluation of nutritive qualities of these products is also briefly discussed.

Infection and control of diseases in fruits and vegetables, ripening of fruits - its regulation (delaying and hastening), critical steps during processing of fruits, potatoes, tomatoes, grapes etc for development of ready-to-eat types of products are discussed in Chapter 4 on Fruits and Vegetables Technology. Pros and cons of different methods of freezing in terms of cost and quality retention and also methods of dehydration are briefly presented.

Technologies of animal and marine foods are discussed in Chapters 5 and 6. India ranks first in milk production in the world and Dairy Industry is well established in India. Success of co-operative dairying, operation flood, milk processing, manufactured products / by-products such as butter, *channa*, cheese, ghee, ice creams etc are well covered along with emphasis on the role of process parameters on quality retention and elimination of spoilage / pathogenic microbes associated with milk and milk products.

Very brief accounts on freezing / chilling, curing / smoking, dehydration, canning, preservation etc of meat, fish, poultry and egg are covered as also their speciality products and by-products. Changes in microbiological, physico-chemical, biochemical as well as sensory quality attributes due to processing and storage are also presented. Highlighted are the aspects of microbial and nutritive qualities of flesh foods as well as egg products.

"Food Additives" is an important chapter (Chapter 7) providing information quite often sought after by the food processors. Role of additives as preservatives, emulsifying / stabilizing / thickening / sequestering / buffering / bleaching / maturing / colouring / flavouring / foaming / anti-caking / anti-foaming / leavening / firming / clarifying agents is discussed. There seems to be lack of updated knowledge on use of certain compounds as additives. For example, benzoic, propionic and sorbic acids and their salts have restricted the use in food products and use of MSG is restricted to 1% (as total glutamates) and is not permitted in infant foods. These chemicals are not GRAS substances as mentioned by the authors. BHT is not allowed as an antioxidant. More care should have been exercised in collecting updated information on food additives by the authors.

Some aspects of extruded foods and food irradiation are included in Chapters 8 and 9, respectively. Design and functional aspects, kind of extruders, factors affecting extrusion process, quality and use of extruded foods, merits and demerits of extrusion technology as well as kinds of ionizing radiations and their applicability in food processing, mechanism of action, preservation of seafoods, spices, onions, potatoes and others, delaying of ripening in mangoes and bananas, radiation dosage requirements and safety of irradiated foods are explained.

Newer developments in food packaging have taken place due to industrialization and urbanization and plastic packaging is becoming more popular. Objectives, functional qualities of varieties of flexible films and their laminates, comparative properties, retention of nutritive qualities in packaged foods and packaging considerations of selected foods such as bakery and dairy products, dehydrated / frozen / convenience / thermally processed foods, sweets and fried nuts, coffee / tea, fruits and vegetables, fats / oils and spices are summarized in Chapter 10.

Authors should have preferably used kg and °C as units to express weight and temperature, respectively throughout this book, instead of sometimes expressing them in lbs and °F. Inadequate details are given for books / journals cited under "Further Reading" at the end of each chapter and no references are cited for the last two chapters.

Many universities in India have started Food Science and Technology, as well as Food and Nutrition courses in Graduate and Post-graduate levels and this book is a valuable guide for students and teachers. The authors deserve sincere appreciation for their efforts in consolidating the latest knowledge available on all aspects of food processing and preservation principles and in bringing out this timely publication.

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